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From the Zoophysiological Institute University of Lund Sweden

The Effect of Estrogenic Hormones on Cell Division and Growth in Some Tissues of Mice

By

INGA PETTERSSON

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Abstract

PETTERSSON I *The effect of estrogenic hormones on cell division and growth in some tissues of mice* Acta physiol scand 1962 *55* 1—10 — The effect of estrogens upon cell division in genital and non genital tissues of mice has been investigated the non genital tissue in vivo as well as in vitro the genital tissue in vivo In the in vivo experiments an increased mitotic index depending upon an increased prophase number was observed from what it may be concluded that there is a real mitotic activation In the in vitro experiments it was observed that the effect of estradiol upon cell division depended upon the concentration in the medium Low concentration caused increased mitotic activity high concentrations on the other hand caused a metaphase block A difference in response to estrogens of genital and non genital tissue as well as a different effect upon tissue in vivo and in vitro have been observed

The estrogens have been considered as growth hormones with particular target organs because of their effect upon the female genital organs It is possible however that these hormones affect other tissues as well BULLOUGH who has made extensive investigations concerning the influence of estrogens upon cell division in most mammalian tissues found that these hormones caused an elevation in the mitotic index in genital as well as non-genital tissue (BULLOUGH 1940—46) AGRELL (1955 1956) on the other hand found that estradiol in high concentration had a c mitotic effect upon sea urchin

embryos It is just possible that the elevation in mitotic index found by BULLOUGH is only apparent *i e*, *it is caused by a metaphase inhibition* Another opinion concerning the effect of estrogens has been expressed by DRASHER (1952) who considers that estrogens do not cause a cell multiplication even in genital organs as she was unable to find any increase in the DNA content in the whole uterus from animals which had been under the influence of estrogens compared with animals which were in diestrus or castrated

The different results which among others the above mentioned investigators had obtained indicate that the problem is very complicated Some of the questions to be raised concerning the influence of estrogenic hormones upon cell division and growth are Do these hormones act as mitosis stimulating or mitosis inhibiting agents? Do the hormones influence genital and non genital tissue in the same way? Do they have the same effect upon tissues in vivo and vitro?

The author has chosen to approach the problem by investigating not only the mitotic index but also the mitotic spectrum The effect of estrogens upon genital and non genital tissue from the same animal has been compared In order to find out whether estradiol affects tissues in different ways in vivo and in vitro tissue culture experiments have been performed The tissue cultivated in vitro was one of those investigated in the in vivo experiments

Material and Methods

The animals used in these experiments were mice from the C3H strain with the exception of the male mice used for the investigation concerning the influence of estradiol upon the blood picture The latter were of an albino strain The animals were kept at a temperature of + 20 C The room was illuminated artificially the light being regulated automatically to give 12 hours of light a day The mice were fed with mice cakes from Konsum Polymin vitamin solution (Ewos) was added to the drinking vater The adult mice used in the experiments were 3—5 months old The weight of the intact and castrated females was about 25 g the weight of the male mice of albino strain were 25—30 g The infant mice used here were 4 weeks old and their weight was 8—10 g Among these infant animals no vaginal opening was observed until the animal was 5 weeks old

The tissues chosen for investigations were uterine epithelium and red bone marrow *The uterus of the mouse is very suitable for investigations of cell division and cell* multiplication In a transverse section the different tissues of the organ form nearly concentric circles and the organ is small enough to enable cell counts in order to obtain an approximate conception of the alteration in the cell number under estrogenic influence The red bone marrow is suitable for investigations because it is a tissue where cell production is going on continually and which has a high mitotic index Further this tissue is suitable for squash preparations and therefore it is easy to count the cells and to calculate the mitotic index as the number of mitoses per thousand cells instead of counting the number of mitoses per length or surface unit a method which must be considered as less accurate Finally the bone marrow is easy to keep in tissue culture which made it possible to compare the effect of estrogens in vivo and in vitro upon the same tissue

The effect of endogenous hormones was studied upon tissues from females killed at different times during the estrus cycle In these experiments only such female mice were used which had shown three regular cycles The cycles were followed with vaginal smears

The hormones used for administration to castrated and to infant mice were Estradiol (Pharmacia) and Poly Estradiol Phosphate (Leo) In the following the polyestradiol phosphate will be written as P E P The preparation first mentioned was us d in order to study the effect of a single dose the latter preparation in order to stud / the depot effect The hormones were given intraperitoneally suspended in physiological NaCl solution The doses given per gram body weight were for the infant females 1 for the castrated females 2 and for the adult males 3 5 μg A test series had shown that the dose given to the infant animals was 5 to 10 times the threshold dose In this test series the frequency of vaginal openings was studied The doses given to the adult mice had been tested upon castrated females It was observed that cornified cells ap pered in the vagina already when only 1 μg per animal was given The effect upon cell division in the bone marrow did not appear until 5 μg per animal were given. The same response was observed in the bone marrow when doses from 5 to 100 μg per animal were given

When the tissues were to be taken for preparation groups of animals were killed at the same hour of the day in order to eliminate the diurnal variation in mitotic ac tivity found by BULLOUGH (1948)

One of the experimental series was performed in order to see whether there is any correlation between an alteration in the mitotic activity in the bone marrow and the peripheral blood picture The animals used in this experiment were adult male mice Groups of 4—6 animals were killed on different days following administration of one single dose of estradiol Immediately before they were killed blood samples were taken from their tail tips White blood cell counts were performed and blood smears were prepared for differential counting of the white cell In order to investigate a possible alteration in the erythropoesis blood smears were stained for counting of reticulocytes About 10 days before killing the animal the normal blood picture had been investigated by performing the same sort of blood tests before administration of estradiol In this way the woundhealing effect upon the blood picture was eliminated

In order to prepare the bone marrow for examination of the mitotic activity the animals were anesthetized with ether and the bone marrow was removed from sternum and fixed in Carnoy s fixation medium Squash preparations were made from the bone marrow specimens and the preparations were stained in Ehrlich s hæmatoxylin For determining the mitotic index and spectrum 2 000 cells were counted The uterus was removed from the same animal from which the bone marrow had been prepared and the organ was fixed in Carnoy and embedded in paraffin wax and cut into sections of 6 μ The sections were stained with Gomory s hæmatoxylin (MELANDER and WING STRAND 1952) For determining the mitotic index and spectrum in the uterine epithelium 4—6 000 cells were counted

For the tissue culture experiments bone marrow from adult male mice was used The tissue p eces were placed on small coagulates made up of cock plasma and embryo extract from chi k embryos 8—10 days old The tissue was cultivated in carell bottles at a temperature of 38 C The fluid medium was composed of denatured horse serum and Tyrode s solution The incubation medium containing estradiol v as prepared in the following way The hormone was eluted in a small amount of ethyl alcohol and Tyrode s solution was added The suspension thus prepared was mixed with serum 1 1 Samples of tissue were removed from the bottles at 2—3 hour intervals during 20—24 hours The mitotic index and spectrum were determined as in the in vivo experiments

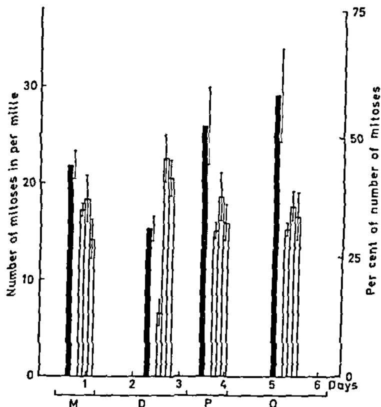


Fig 1 Mitotic index and mitotic spectrum of the bone marrow of female mice during different stages of the estrous cycle On the line beneath the abscissa the different stages are marked The black columns represent the mitotic index and the unfilled columns represent the different stages of mitosis from the left to the right Prophase metaphase ana and telophase The mean errors are marked as thin lines Every group of columns represents the mean values of 4 –5 animals

Results

Fig 1 shows the variation in mitotic index and spectrum of bone marrow of female mice during the estrus cycle The lowest mitotic index and prophase number was found during diestrus In the uterine epithelium the mitotic index was highest during this phase of the cycle In proestrus the proliferation of the epithelium was interrupted The mitotic spectrum during this stage of the cycle showed a high prophase number (64 %) which is due to the fact that the dividing cells in other stages of mitoses are necrotic During estrus no mitoses were to be seen Among the animals used for this experiment the mean length of the cycle was found to be 5 5 days The animals were in diestrus during 33 9 % in proestrus during 14 9 % in estrus during 36 5 % and in metestrus during 36 5 % of this time

Fig 2 shows the alteration in mitotic activity in the bone marrow of infant mice after administration of I E P The mitotic index and prophase number

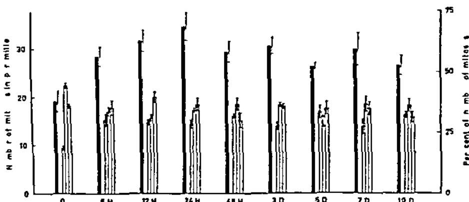


Fig 7 Mitotic index and mitotic spectrum in the bone marrow of infant female mice after inject on of 10 μg P E P per animal E ery group of columns represents the mean value of 5 m ce

increased only 6 hours after the hormone injection The case was the same if estradiol was injected A difference was observed between the mitotic reaction after injection of estradiol and after that of P E P In the first case the mitotic index and spectrum returned to those of the controls already after 24 hours but in the second case the mitotic index and prophase number were high during 10 days During the same time the estrogenic activity of P E P was indicated by cornified cells in the vaginal smears

In the uterine epithelium of the infant mice the mitotic activity is very low When this tissue was investigated in the infant mice mentioned above an increased mitotic activity was observed culminating 24 hours after the in jection of estradiol and P E P respectively In the uterine epithelium of the animals which had been injected with estradiol the mitotic index rose from 2 to 19 2 °/ and in the animals which had received P E P from 2 to 10 3 °/₀₀ The rise in the mitotic index was due to rise in the prophase number in both cases In the animals which had been injected with estradiol the uterine epithelium in one other group killed after 48 hours was investigated The mitotic index and the prophase number had returned to those of the controls The case was the same in the animals which had got P E P and which were investigated 4 days after the injection

The results from cell counts on uterus sections from animals killed at dif ferent times during the estrus cycle showed that the number of cells in the endometrium from animals in estrus and metestrus was about twice that of those in diestrus and proestrus Even in the infant females which had re ceived P E P the cell number of the endometrium had increased as compared with the control animals The cell multiplication seems to occur mainly in the epithelium These cell counts however were performed on a limited material and therefore the results obtained are to be considered as preliminary

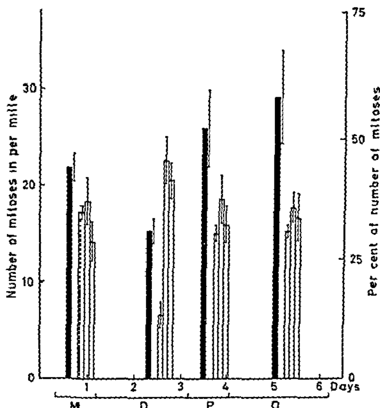


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Fig 2 shows the alteration in mitotic activity in the bone marrow of infant mice after administration of P E P The mitotic index and prophase number

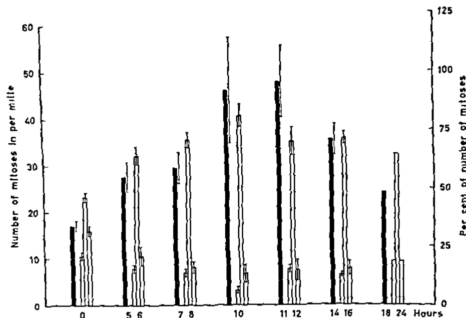


Fig 4 Mitotic index and mitotic spectrum in bone marrow cultivated in a medium containing 10 μg estradiol per ml

Fig 4 presents the mitotic activity of bone marrow cultivated in a medium containing 10 μg estradiol per ml From this diagram it is seen that the increased mitotic index depends upon an increase in the metaphase number *i e* a metaphase block is observed

Discussion

With regard to the question whether there is a real cell multiplication following estrogenic influence the author s results from the in vivo experiments confirm the results obtained by BULLOUGH (1940—46) The increase observed in the mitotic index depends upon an increase in the prophase number From this result it can be concluded that there is a real mitotic activation It is known about most mitotis inhibiting substances that they have effect either upon the metaphase causing a metaphase block or upon the preprophase causing abnormal cells and necrotic nuclei (DUSTIN 1947) In the tissues investigated here it was the prophase number which was increased and no necrotic nuclei were seen immediately after the estrogen induced mitotic activity had started A real cell multiplication is also suggested by the cell number of the uterine epithelium during estrus and after estrogen administration Thes results seem to be contradictory to those obtained by DRASHER (1952) The fact that she was not able to find any increase in the DNA content

of the whole uterus after estrogen stimulus does not exclude a cell multiplication in one of the tissues of this organ In connection with the investigation of mitosis in the bone marrow it might be of interest to see whether there is any correlation between the increased mitotic activity in this tissue and the peripheral blood picture The result from the experimental series performed for this purpose is not sufficiently clear to settle this question The erythropoesis did not seem to be affected and the sharp drop in the white cell count may as well be an unspecific stress effect

Evidently endogenous as well as exogenous estrogenic hormones cause a real mitotic activation the exogenous ones when given in vivo AGRELL (1955) on the other hand, found that estradiol caused a metaphase block in sea urchin embryos Therefore the question ought to be raised as to whether the estrogens act in another way in vitro than in vivo The author has not been able to see any inhibitory effect of estradiol *in vivo* in spite of giving un physiologically high doses Not even KNAKE (1950 1951) giving still higher doses observed any deviation from the normal mitotic activity The results from the author s in vitro experiments however show that estradiol in low concentration has a mitosis-stimulating effect but inhibits in high concentration. A metaphase block was observed when the concentration of estradiol *in the medium was ten times the mitosis-stimulating concentration* AGRELL (1955) found no mitotic activation when using low concentrations of estradiol in the cultivation medium. This may depend upon the fact that he used early embryos and in such material the mitoses are at maximal frequency and no further activation is possible It may be mentioned that the results from an investigation of the effect of estradiol upon regeneration of planarian worms performed by AGRELL and WIMAN (1957) indicate a mitotic activation as well as at the same time a c mitotic effect It seems that the in vitro effect of estradiol upon mitoses may be an activating one when low concentrations are used and an inhibiting one when high concentrations are used A special case is the embryonic tissue which seems to be refractory to further mitotic stimulus

The reason for the failure of estradiol to inhibit mitosis in vivo may be that it is rapidly metabolized in the organism and that the metabolic products are less estrogenic than estradiol (PASCHKIS and RAKOFF 1950) Estradiol is found to be inactivated in different organs especially in the liver (HELLER 1940)

Another question finally is whether estrogens have the same effect upon genital and non-genital tissue and whether the former effect is only an accentuation of a general growth promoting effect *Some difference in the* effect of these hormones upon the two kinds of tissues investigated here have been observed. The author found that the response to estrogen administration appeared earlier in the bone marrow than it did in the uterine epithelium. During the estrus cycle the increased mitotic activity appeared during dif

ferent phases of the cycle Another difference in the response to estrogens in the two kinds of tissue was the duration of the increased mitotic activity If estradiol was given to infant or to castrated female mice the mitotic wave thus induced in the bone marrow passed after 48 hours However, when P E P was given the mitotic index and the prophase number were high as long as the vaginal smears contained cornified cells i e about 10 days In the uterine epithelium however the increased mitotic activity did not seem to last any longer after administration of P E P than after that of estradiol The difference in the duration of the response to P E P in the bone marrow and in the uterine epithelium may depend upon the fact that the estrogenic hormones stimulate cell division in non genital tissues but in genital tissues cell division as well as cell differentiation is stimulated DRASHER (1952) has found a considerable increase in the RNA content of the uterus after endogenous or exogenous estrogenic stimulus Further growth of the different cell organelles of endometrial cells was observed which at last led to a breakdown of the physiological balance of the cells For that reason the tissue becomes necrotic some time after the increased mitotic activity has started and consequently the mitotic activity is interrupted

It is not fully known how the estrogens affect the tissues BULLOUGH (1950) is of the opinion that estrogens act in two ways By forcing more cells into mitoses and by shortening the time for each mitose to be completed He explains the effect first mentioned in that the estrogens activate the mechanism for the entrance of glucose into the cells Thus the cells would get the energy necessary for the starting of mitoses To the latter effect he is not able to give any explanation

AGRELL and PERSSON (1956) have given another explanation to the mitosis stimulating as well as the mitosis inhibiting effect of estradiol They found that estradiol caused the DNA to become separated from the protein and thus be more exposed to the action of DNA se The inhibiting effect should be particularly marked in embryonic cells which have much cytoplasmic DNA A breakdown of this cytoplasmic DNA may be followed by a destruction of plasma structures among them the aster and spindle In adult tissues where the cells do not contain so much cytoplasmic DNA it might be that a small part of the nuclear DNA may be separated from its protein the DNA thus becoming more easily metabolized In that way we may get a stimulation of the DNA synthesis with a possible cell multiplication as a consequence

From what is said above we may conclude that the estrogens seem to affect genital and non genital tissues and organs in different ways How these hormones as well as most other hormones act at the cellular level is still obscure The author has found some difference in the action of estrogens in vivo and in vitro Some of the hypotheses on the mode of action of estrogens have been related and the author has tried to discuss some of the contradictory results obtained by different investigators

The investigation was facilitated by grants from the Royal Physiographical Society and from the University of Lund For valuable technical assistance I want to thank Mrs EDA GUSTAFSON

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From the Department of Pharmacology Karolinska Institutet Stockholm 60 Sweden

Observations on Anaphylactic and Compound 48/80-Induced Histamine Release from Guinea-Pig and Rat Lung Tissue in Vitro

By

Bertil Diamant

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Abstract

Diamant B *Observations on anaphylactic and compound 48/80 induced histamine release from guinea pig and rat lung tissue in vitro* Acta physiol scand 1962 *55* 11—25 — The effect of oxygen lack on anaphylactic histamine release from sensitized guinea pig and rat lung tissue *in vitro* has been investigated and found to consist of pronounced inhibition provided that the incubation medium (phosphate buffer) was devoid of glucose In the presence of glucose this inhibition was overcome progressively with rising concentrations of glucose Under oxygen the anaphylactic histamine release from guinea pig lung tissue was also enhanced by the presence of glucose By comparing anaphylactic histamine release from guinea pig lung tissue and Compound 48/80 induced histamine release from rat lung tissue in glucose containing phosphate buffer and Tyrode solution respectively it has been demonstrated that pH changes produced by gassing the Tyrode solution with oxygen and nitrogen have obscured the true effect of nitrogenation in earlier investigations performed in glucose containing Tyrode solution

It is generally agreed that anaphylactic histamine release *in vitro* from guinea pig lung tissue involves energy requiring reactions This conclusion is based on the findings that oxygen lack (Parrot 1942 Mongar and Schild 1957 Chakravarty 1960 Moussatche and Provoust Danon 1960 Yamasaki Muraoka and Endo 1960) and inhibitors of oxidative phosphorylation (Mongar and Schild 1957 Moussatche and Provoust Danon 1958) depress anaphylactic release of histamine These findings have been interpreted as a

dependence on intact oxidative metabolic pathways for the energy production furnishing the anaphylactic histamine releasing reaction in the guinea pig.

In contrast, both anaphylactic and Compound 48/80-induced histamine release *in vitro* from rat lung tissue was reported to be uninfluenced by oxygen lack (CHAKRAVARTY 1960). This suggested that the energy production could be furnished anaerobically in this species. In support of this hypothesis CHAKRAVARTY (1959) put forward the observation of Diamant, i.e. that as long as the incubation medium contained glucose, the histamine release elicited from rat lung tissue by extracts of *Ascaris suis* appeared despite oxygen lack. In the absence of glucose, on the other hand, the histamine release was markedly inhibited (DIAMANT 1961). In the rat, glucose and oxygen lack were both found to have a similar effect on histamine release as well as on mast cell degranulation when elicited by the polymer amine Compound 48/80 (DIAMANT and UVNÄS 1961).

In the present investigation, the anaphylactic histamine release *in vitro* from rat and guinea pig lung tissue has been studied with respect to the effect of glucose under aerobic and anaerobic conditions. Furthermore, in order to evaluate the varying results reported in earlier investigations as to the effect of oxygen lack, anaphylactic histamine release from guinea pig lung tissue has been compared with that elicited by Compound 48/80 from rat lung tissue in different incubation media.

Material and Methods

Guinea pigs (male and female, 250—350 g) and rats (male and female, 200—300 g) were sensitized by injection of crystallized egg albumin. Guinea pigs were injected subcutaneously with 0.1 g and intraperitoneally with 0.1 g, followed 3 days later by a s. c. injection of 0.1 g. Rats were injected s. c. with 0.1 g of egg albumin together with 1 ml of *H. pertussis* vaccine (2×10^{10} bacilli per ml) followed 3 days later by another s. c. injection of 0.1 g of egg albumin. The sensitized guinea pig lungs were used for *in vitro* anaphylactic reactions 3—25 weeks after the first sensitizing dose, and the sensitized rat lungs 3—8 weeks after it.

The lungs of the sensitized animals were treated and incubated as described in detail earlier (DIAMANT 1961) with modifications according to DIAMANT and UVNÄS (1961). In the experiments where the effect of Compound 48/80 was investigated, non-sensitized rat lungs were used and treated by the same method.

Two different solutions were used for preparation and incubation of the lung tissue. (1) A solution (denoted in the following as phosphate buffer) containing NaCl (154 mM), KCl (2.7 mM) and $CaCl_2$ (0.9 mM) buffered with 10 % v/v Sørensen phosphate buffer (67 mM). The initial pH of the phosphate buffer ranged from 7.1 to 7.2 in the single experiments. (2) Tyrode solution containing NaCl (137 mM), KCl (2.7 mM), $CaCl_2$ (1.8 mM), $MgCl_2 \cdot 6H_2O$ (1.0 mM), $NaHCO_3$ (11.9 mM) and $NaH_2PO_4 \cdot H_2O$ (0.4 mM). The initial pH of the Tyrode solution ranged from 7.7 to 8.3 in the single experiments. Neither the phosphate buffer nor the Tyrode solution contained glucose, i.e. during preparation the lung tissue was treated with a glucose free solution in all experiments. The interval between sacrificing the animals and the incubation of the lung tissue amounted to 1 1/2—3 hours.

Table I Spontaneous histamine release from rat and guinea pig lung tissue in phosphate buffer under the influence of oxygen nitrogen and glucose (5 6 mM) Histamine release computed in of total histamine content mean and standard error of the mean Bracketed figures denote the number of experiments

Species	Spontaneous histamine release			
	O + glucose	N + glucose	O	N
Rat	8 0 ± 0 69 (10)	6 6 ± 0 40 (23)	6 7 ± 0 53 (13)	7 0 ± 0 45 (28)
Guinea pig	1 1 ± 0 14 (5)	1 3 ± 0 09 (6)	1 0 ± 0 23 (4)	1 0 ± 0 13 (6)

In some experiments in which the effect of phosphate buffer and Tyrode solution on histamine release was investigated the lung tissue was cut into coarse pieces in physiologic sodium chloride solution After thorough mixing it was divided into two parts by weighing one part was then treated and incubated in phosphate buffer and the other in Tyrode solution

The gaseous phases used during incubation usually consisted of commercially pure oxygen or nitrogen In some experiments however gas mixtures composed of oxygen or nitrogen (93 5 %) together with CO_2 (6 5) were used

The lung tissue was exposed to crystallized egg albumin (1 mg/ml) or Compound 48/80 (35 μg/ml) for 20 min in all experiments After withdrawal of the incubation fluids their pH was determined electrometrically All concentrations of substances given in the text refer to the final concentration in the incubation fluid

The remaining histamine of the lung tissue after incubation was extracted by heating to 100 C for 10 min This was done in a slightly acid medium (pH 3—4) by addition of N HCl in all experiments where the lung tissue was extracted in Tyrode solution (in order to avoid breakdown of histamine at alkaline pH) as well as when guinea pig lung tissue was extracted in phosphate buffer (in order to inactivate substances interfering with the histamine assay on the isolated guinea pig ileum) In the experiments where the effect of phosphate buffer and Tyrode solution was investigated simultaneously all lung samples were extracted with a phosphate buffer All acidified extraction samples were neutralized before histamine assay

Histamine was assayed on atropinized guinea pig ileum At least 4 single contractions of each sample were compared with a known histamine dihydrochloride solution In random samples mepyramin completely inhibited the contractions

Unless otherwise stated the histamine release was computed in of the total histamine content of the lung tissue The amount of histamine found in controls without antigen or Compound 48/80 (denoted as *spontaneous histamine release*) was *deducted from all* values given in the text

Results

Spontaneous histamine release from guinea pig and rat lung tissue

The spontaneous histamine release from guinea pig and rat lung tissue in phosphate buffer under the influence of oxygen nitrogen and glucose is shown in Table I No significant difference between the histamine release under the various metabolic conditions was observed within the same species A significant difference was on the contrary noted in a comparison between guinea pig

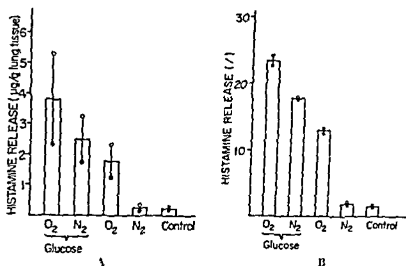


Fig 1 (A and B) Effect of oxygen nitrogen and glucose (5 6 mM) on anaphylactic histamine release from guinea pig lung tissue in phosphate buffer Control = spontaneous histamine release (not deducted from antigen induced histamine release) Filled and open circles represent individual values in two experiments
A µg of histamine base released from 1 g of wet lung tissue
B Histamine release computed in % of total histamine content

and rat lung tissue under the same metabolic conditions Thus the spontaneous histamine release from guinea pig lung tissue amounted to only 14—20 % of that from rat lung tissue This is noteworthy since the histamine content of *guinea pig lung tissue in the various experiments usually was 2—3 times that* of rat lung tissue

Effect of glucose on anaphylactic histamine release from guinea pig and rat lung tissue in phosphate buffer

The histamine release varied considerably in single experiments under uniform experimental conditions when expressed as µg of histamine base released from 1 g of wet lung tissue This is shown in Fig 1 A in which the same batch of sensitized guinea pigs was used and the interval between the two experiments was 2 days When the histamine release was calculated in % of the total histamine content of the lung tissue (Fig 1 B) the variation was markedly diminished Consequently it was considered most adequate in the following to use the percentage histamine release in order to eliminate at any rate those experimental variations due to differences between the total histamine content of the lung tissue in individual animals

In the absence of glucose oxygen lack inhibited anaphylactic histamine release from guinea pig lung tissue Fig 1 A and B) This inhibition was, however, markedly counteracted by the presence of 5 6 mM glucose (1 mg/ml) In oxygenated phosphate buffer 5 6 mM glucose also produced activation of histamine release Quantitative data from 7 separate experiments on guinea

Table II Anaphylactic histamine release from guinea pig lung tissue in phosphate buffer under the influence of oxygen nitrogen and glucose (5.6 mM) Histamine release computed in % of total histamine content Spontaneous release deducted from all values Bracketed figures denote the pH of the phosphate buffer after incubation

Experiment no	Initial pH of phosphate buffer	Histamine release			
		O + glucose	O	N + glucose	N
1	7.1	38.7 (6.8)	27.0 (7.0)	20.2 (6.8)	0.0 (7.0)
2	7.2	29.9 (6.9)	19.3 (7.1)	23.4 (6.9)	0.3 (7.0)
3	7.2	34.5 (6.9)	25.1 (7.0)	26.0 (6.8)	2.6 (7.0)
4	7.2	36.1 (6.9)	29.7 (7.1)	27.2 (6.8)	2.7 (7.0)
5	7.2	38.1 (6.9)	23.8 (7.0)	34.5 (6.8)	7.9 (7.0)
6	7.2	20.9 (6.9)	11.6 (7.2)	16.2 (6.9)	0.3 (7.1)
7	7.2	22.6 (6.9)	11.4 (7.1)	16.3 (6.9)	0.4 (7.1)
Mean and standard error of mean		31.5 ± 2.8	21.1 ± 2.8	23.4 ± 2.5	2.0 ± 1.1
Significance of difference of mean from O + glucose (*t* test)			P = 0.05–0.01	P = 0.05–0.01	P = < 0.001

Table III Effect of glucose (5.6 mM) on anaphylactic histamine release from rat lung tissue in phosphate buffer under nitrogen Histamine release computed in % of total histamine content Spontaneous release deducted from all values Bracketed figures denote the pH of the phosphate buffer after incubation.

Experiment no	Initial pH of phosphate buffer	Histamine release	
		N + glucose	N
1	7.2	30.4 (7.0)	< 4.0 (7.1)
2	7.2	31.5 (7.0)	0.0 (7.2)
3	7.2	14.0 (6.9)	0.3 (7.0)
4	7.2	29.2 (6.9)	1.1 (7.0)
5	7.2	20.3 (6.9)	0.0 (7.1)
	Mean	25.1	< 1.1

pig lung tissue are given in Table II Histamine release was found to be optimal under oxygen in the presence of 5.6 mM glucose amounting on the average to 32 % of the total histamine content Under oxygen in the absence of glucose 21 % was released The histamine release under nitrogen in the absence of glucose averaged 2 % of the total histamine content it was raised to 23 % by addition of 5.6 mM glucose Table II shows in addition the significance of the differences from the optimal histamine release (*i e* with oxygen and glucose) The pH of the phosphate buffer before and after incubation with the lung

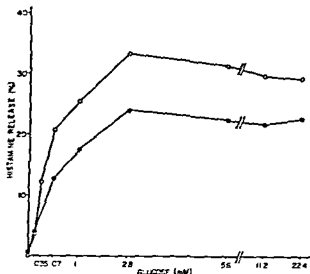


Fig 2 Effect of various concentrations of glucose on anaphylactic histamine release from rat (—o—o—) and guinea pig (—•— —) lung tissue in nitrogenated phosphate buffer Histamine release computed in % of total histamine content. Spontaneous histamine release deducted from all values

tissue under the various experimental conditions is also recorded in Table II In every experiment the decrease in pH of the phosphate buffer after incubation was somewhat greater in the presence of glucose Thus the pH was usually 0 2 units lower than that of phosphate buffer incubated in the absence of glucose

The effect of 5 6 mM glucose on the histamine release from sensitized rat lung tissue under oxygen lack is shown in Table III (5 separate experiments) The average histamine release amounted to about 1 % of the total histamine content in the absence of glucose as compared to 25 % in the presence of glucose The fall in pH of the phosphate buffer after incubation was slightly greater 0 1—0 2 units in the presence of glucose than in its absence.

The effect of different concentrations of glucose on the anaphylactic histamine release from guinea pig and rat lung tissue in phosphate buffer and under oxygen lack is shown in Fig 2 The histamine release increased progressively with rising glucose concentration up to 2 8 mM (0 5 mg ml) in both species An increase in glucose concentration above 2 8 mM had no greater effect on histamine release Judging by the curves in Fig 2 0 25 mM (0 045 mg ml) is about the lowest concentration that would produce an appreciable increase in histamine release in both species as compared to that in nitrogenated buffer without addition of glucose

Effect of oxygen nitrogen carbon dioxide and glucose on anaphylactic histamine release from guinea pig lung tissue and on Compound 48 80-induced histamine release from rat lung tissue in phosphate buffer and Tyrode solution

There is a considerable discrepancy between the data given by various authors on the inhibition of anaphylactic histamine release under oxygen lack

Table II Effect of oxygen lack on anaphylactic histamine release from sensitized lung tissue as reported in earlier investigations

Investigator	Species	Incubation medium	Glucose present in incubation medium	Histamine release under oxygen lack
PARROT (1942)	Guinea pig	Tyrode solution	*	Inhibition (no values given)
MONGAR and SCHILD (1957)	Guinea pig	Tyrode solution	5 6 mM	32 (range 6–55) % of histamine release in aerated medium
CHAKRAVARTY (1960)	Guinea pig	Tyrode solution	*5 6 mM	14–29 % of histamine release in oxygenated medium
MOUSSATCHÉ and PROVOUST DANON (1960)	Guinea pig	Ringer Barron solution	0	0 % of histamine release in oxygenated medium
YAMASAKI MURAOKA and ENDO (1960)	Guinea pig	Krebs-Ringer solution	0	0 % of total histamine content released
CHAKRAVARTY (1960)	Rat	Tyrode solution	*5 6 mM	No inhibition

* Personal communication.

Earlier reports on the effect of oxygen lack on the histamine release from sensitized guinea pig and rat lung tissue are summarized in Table II

It can be seen that anaphylactic histamine release from guinea pig lung tissue was incompletely and variably inhibited by oxygen lack in a medium containing glucose (Tyrode solution) This is in contrast to the complete inhibition noted in incubation media devoid of glucose (Krebs Ringer and Ringer Barron solution) It is also evident that the histamine release from the sensitized lung tissue of the rat — unlike that of the guinea pig — was found to be uninhibited in Tyrode solution despite oxygen lack CHAKRAVARTY (1960) also reported that Compound 48/80-induced histamine release from rat lung tissue in Tyrode solution was uninfluenced by oxygen lack

I therefore considered it of interest to investigate whether the varying effect of anoxia on histamine release in Tyrode solution observed in earlier studies was due to the glucose present or whether some other factor might be involved Consequently it was decided to compare the histamine release in phosphate buffer and in Tyrode solution in order to evaluate the differences produced by the incubation media

The effect of 5 6 mM glucose on the anaphylactic histamine release from guinea pig lung tissue under oxygen and nitrogen in phosphate buffer and in Tyrode solution is shown in Fig 3 In oxygenated glucose containing

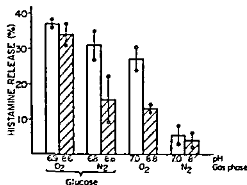


Fig 3 Effect of oxygen nitrogen and glucose (5 6 mM) on anaphylactic hista mine release from guinea pig lung tissue in phosphate buffer (unshaded blocks) and Tyrode solution (shaded blocks) Mean values of two experiments are shown open and filled circles represent the individual values in each experiment. The final pH of the incubation medium is given below each block Initial pH of incubation media phosphate buffer 7.2 Tyrode solution 8.2 Histamine release computed in of total histamine content Spontaneous release deducted from all values.

phosphate buffer the release averaged about 37 % of the total histamine content against about 34 % in oxygenated glucose containing Tyrode solu tion In correspondingly nitrogenated glucose containing incubation media the histamine release averaged 31 % and 15 % respectively In oxygenated glucose free phosphate buffer 27 % was released against 13 % in oxygenated glucose free Tyrode solution whereas 6 % and 4 % respectively, was released in correspondingly nitrogenated glucose free media It was further noted that both oxygenation and nitrogenation of the Tyrode solution during incubation raised the final pH to 8 6—8 9

It is evident that the difference between the histamine release in the two incubation media was negligible under oxygen in the presence of glucose In nitrogenated glucose-containing Tyrode solution however, the histamine release markedly decreased as compared to that in phosphate buffer under equal experimental conditions This difference is further shown in Table V which gives the individual values in 5 experiments on the histamine release from sensitized guinea pig lung tissue under oxygen lack in glucose containing Tyrode solution and phosphate buffer respectively together with the pH of the incubation media before and after incubation It is seen that the histamine release in Tyrode solution ranged from 10 % to 63 % of that in phosphate buffer in the various experiments This is in contrast to the small variations within single experiments (exp 4 and 5 Table V) The pH of the phosphate buffer which was initially 7 2 in all experiments fell during incubation to 6 8—6 9 In Tyrode solution on the contrary the pH rose to 8 8 in every experiment despite an initial range of 7 7—8 3

It is obvious from the reports of MONGAR and SCHILD (1958) and CHAKRAVARTY (1960) that anaphylactic histamine release from guinea pig lung tissue is not optimal at pH 8 8 These workers used Tyrode solution as incubation medium when studying the influence of oxygen lack on histamine release Consequently changes in the pH of the solution towards the alkaline side due to the experimental conditions might have obscured the true effect of oxygen lack in these investigations

Table 1 Anaphylactic histamine release from guinea-pig lung tissue under nitrogen in phosphate buffer and Tyrode solution in the presence of glucose (5 6 mM) Histamine release computed in of total histamine content Spontaneous release deducted from all values Bracketed figures denote the pH of the solutions after incubation Initial pH of incubation media phosphate buffer 7 2 Tyrode solution 7 7—8 3 Variations in histamine release in double and triple tests are shown (experiments 4 and 5) $\frac{B}{A}\times$ 100 denotes histamine release in Tyrode solution computed in of that in phosphate buffer

Experiment no	Histamine release				
	Phosphate buffer (A)		Tyrode solution (B)		$\frac{B}{A}$ × 100
1	16 3 (6 9)		4 1 (8 8)		25
2	34 5 (6 8)		21 6 (8.8)		63
3	27 2 (6 8)		9 1 (8 8)		34
4	27 1 (6 9)	26 5 (6 9) 27 6 (6 9)	2 8 (8 8)	1 4 (8 8) 4 3 (8 8)	10
5	19 6 (6 8)	20 0 (6 8) 20 1 (6 9) 18 9 (6 8)	7 2 (8 8)	7 4 (8 8) 6 5 (8.8) 7 6 (8 8)	45

In order to test this hypothesis the pH changes of phosphate buffer and Tyrode solution (initial pH 7 2 and 7 8 respectively) were first studied in the absence of lung tissue after exposure to oxygen and nitrogen with and without CO_2 (6 5 %) for 35 min at 37 C After exposure to oxygen and nitrogen without CO_2 the final pH was 7 3 in phosphate buffer and 9 1 in Tyrode solution In the presence of CO the pH fell to 6 5 in phosphate buffer and to 7 1 in Tyrode solution both with oxygen and nitrogen In the absence of lung tissue addition of 5 6 mM glucose did not influence the final pH

In view of these pH changes rat and guinea pig lung tissue respectively (pooled from 3—5 animals) was divided into two parts One part was prepared and incubated in Tyrode solution and the other in phosphate buffer Nitrogen alone or a mixture of nitrogen (93 5 %) and CO_2 (6 5 %) was used during incubation in order to achieve anoxic experimental conditions It is apparent from Figs 4 and 5 that in glucose containing (5 6 mM) Tyrode solution both anaphylactic histamine release from guinea pig lung tissue (Fig 4) and Compound 48/80-induced histamine release from rat lung tissue (Fig 5) were markedly enhanced by CO_2 under oxygen lack Without CO_2 the final pH of the Tyrode solution was 8 7—8 9 in the presence of CO it fell to 7 5—7 6 In glucose containing (5 6 mM) phosphate buffer on the other hand CO_2 instead induced a decrease in histamine release as compared to that under nitrogen without CO_2 In both experiments the final pH of the phosphate buffer was 6 9 without CO_2 whereas it fell to 6 6 in the presence of CO_2 It is further evident that in both

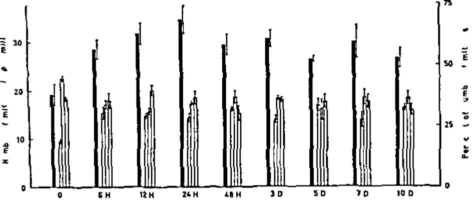


Fig 2 Mitotic index and mitotic spectrum in the bone marrow of infant female mice after injection of 10 μg P E P per animal Every group of columns represents the mean value of 5 mice

increased only 6 hours after the hormone injection The case was the same if estradiol was injected A difference was observed between the mitotic reaction after injection of estradiol and after that of P E P In the first case the mitotic index and spectrum returned to those of the controls already after 24 hours but in the second case the mitotic index and prophase number were high during 10 days During the same time the estrogenic activity of P E P was indicated by cornified cells in the vaginal smears

In the uterine epithelium of the infant mice the mitotic activity is very low When this tissue was investigated in the infant mice mentioned above an increased mitotic activity was observed culminating 24 hours after the injection of estradiol and P E P respectively In the uterine epithelium of the animals which had been injected with estradiol the mitotic index rose from 2 to 19 2 ‰ and in the animals which had received P E P from 2 to 10 3 ‰ The rise in the mitotic index was due to rise in the prophase number in both cases In the animals which had been injected with estradiol the uterine epithelium in one other group killed after 48 hours was investigated The mitotic index and the prophase number had returned to those of the controls The case was the same in the animals which had got P E P and which were investigated 4 days after the injection

The results from cell counts on uterus sections from animals killed at different times during the estrus cycle showed that the number of cells in the endometrium from animals in estrus and metestrus was about twice that of those in diestrus and proestrus Even in the infant females which had received P E P the cell number of the endometrium had increased as compared with the control animals The cell multiplication seems to occur mainly in the epithelium These cell counts however were performed on a limited material and therefore the results obtained are to be considered as preliminary

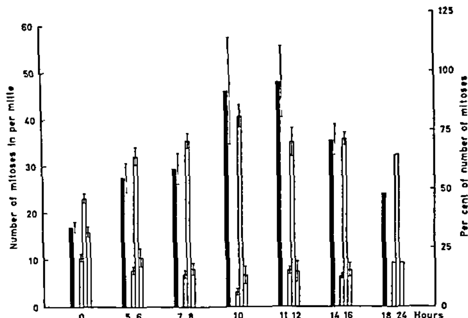


Fig 4 Mitotic index and mitotic spectrum in bone marrow cultivated in a medium containing 10 µg estradiol per ml

Fig 4 presents the mitotic activity of bone marrow cultivated in a medium containing 10 µg estradiol per ml From this diagram it is seen that the increased mitotic index depends upon an increase in the metaphase number i e a metaphase block is observed

Discussion

With regard to the question whether there is a real cell multiplication following estrogenic influence the author s results from the in vivo experiments confirm the results obtained by BULLOUGH (1940—46) The increase observed in the mitotic index depends upon an increase in the prophase number From this result it can be concluded that there is a real mitotic activation It is known about most mitosis inhibiting substances that they have effect either upon the metaphase causing a metaphase block or upon the preprophase causing abnormal cells and necrotic nuclei (DUSTIN 1947) In the tissues investigated here it was the prophase number which was increased and no necrotic nuclei were seen immediately after the estrogen induced mitotic activity had started A real cell multiplication is also suggested by the cell number of the uterine epithelium during estrus and after estrogen administration Thes results seem to be contradictory to those obtained by DRASHER (1952) The fact that she was not able to find any increase in the DNA

of the whole uterus after estrogen stimulus does not exclude a cell multiplication in one of the tissues of this organ In connection with the investigation of mitosis in the bone marrow it might be of interest to see whether there is any correlation between the increased mitotic activity in this tissue and the peripheral blood picture The result from the experimental series performed for this purpose is not sufficiently clear to settle this question The erythropoesis did not seem to be affected and the sharp drop in the white cell count may as well be an unspecific stress effect

Evidently endogenous as well as exogenous estrogenic hormones cause a real mitotic activation the exogenous ones when given in vivo AGRELL (1955) on the other hand found that estradiol caused a metaphase block in sea urchin embryos Therefore the question ought to be raised as to whether the estrogens act in another way in vitro than in vivo The author has not been able to see any inhibitory effect of estradiol in vivo in spite of giving unphysiologically high doses Not even KNAKE (1950 1951) giving still higher doses, observed any deviation from the normal mitotic activity The results from the author s in vitro experiments however show that estradiol in low concentration has a mitosis stimulating effect but inhibits in high concentration A metaphase block was observed when the concentration of estradiol in the medium was ten times the mitosis stimulating concentration AGRELL (1955) found no mitotic activation when using low concentrations of estradiol in the cultivation medium This may depend upon the fact that he used early embryos and in such material the mitoses are at maximal frequency and no further activation is possible It may be mentioned that the results from an investigation of the effect of estradiol upon regeneration of planarian worms performed by AGRELL and WIMAN (1957) indicate a mitotic activation as well as at the same time a c mitotic effect It seems that the in vitro effect of estradiol upon mitoses may be an activating one when low concentrations are used and an inhibiting one when high concentrations are used A special case is the embryonic tissue which seems to be refractory to further mitotic stimulus

The reason for the failure of estradiol to inhibit mitosis in vivo may be that it is rapidly metabolized in the organism and that the metabolic products are less estrogenic than estradiol (PASCHKIS and RAKOFF 1950) Estradiol is found to be inactivated in different organs especially in the liver (HELLER 1940)

Another question finally is whether estrogens have the same effect upon genital and non genital tissue and whether the former effect is only an accentuation of a general growth promoting effect Some difference in the effect of these hormones upon the two kinds of tissues investigated here have been observed The author found that the response to estrogen administration appeared earlier in the bone marrow than it did in the uterine epithelium During the estrus cycle the increased mitotic activity appeared during dif

ferent phases of the cycle Another difference in the response to estrogens in the two kinds of tissue was the duration of the increased mitotic activity If estradiol was given to infant or to castrated female mice the mitotic wave thus induced in the bone marrow passed after 48 hours However when P E P was given the mitotic index and the prophase number were high as long as the vaginal smears contained cornified cells i e about 10 days In the uterine epithelium however the increased mitotic activity did not seem to last any longer after administration of P E P than after that of estradiol The difference in the duration of the respon e to P E P in the bone marrow and in the uterine epithelium may depend upon the fact that the estrogenic hormones stimulate cell division in non genital tissues but in genital tissues cell division as well as cell differentiation is stimulated DRASHER (1952) has found a considerable increase in the RNA content of the uterus after endog enous or exogenous estrogenic stimulus Further growth of the different cell organelles of endometrial cells was observed which at last led to a breakdown of the physiological balance of the cells For that reason the tissue becomes necrotic some time after the increased mitotic activity has started and con sequently the mitotic activity is interrupted

It is not fully known how the estrogens affect the tis ues BULLOUGH (1950) is of the opinion that estrogens act in two ways By forcing more cells into mitoses and by shortening the time for each mitose to be completed He explains the effect first mentioned in that the estrogens activate the mechanism for the entrance of glucose into the cells Thus the cells would get the energy necessary for the starting of mitoses To the latter effect he is not able to give any explanation

AGRELL and PERSSON (1956) have given another explanation to the mitosis-stimulating as well as the mitosis inhibiting effect of estradiol They found that estradiol caused the DNA to become separated from the protein and thus be more exposed to the action of DNA se The inhibiting effect should be particularly marked in embryonic cells which have much cytoplasmic DNA A breakdown of this cytoplasmic DNA may be followed by a destruc tion of plasma structures among them the aster and spindle In adult tissues where the cells do not contain so much cytoplasmic DNA it might be that a small part of the nuclear DNA may be separated from its protein the DNA thus becoming more easily metabolized In that way we may get a stimulation of the DNA synthesis with a possible cell multiplication as a consequence

From what is said above we may conclude that the estrogens seem to affect genital and non genital tissues and organs in different ways How these hor mones as well as most other hormones act at the cellular level is still obscure The author has found some difference in the action of estrogens in vivo and in vitro Some of the hypotheses on the mode of action of estrogens have been related and the author has tried to discuss some of the contradictory results obtained by different investigators

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From the Department of Pharmacology Karolinska Institutet Stockholm 60 Sweden

Observations on Anaphylactic and Compound 48/80-Induced Histamine Release from Guinea Pig and Rat Lung Tissue in Vitro

By

Bertil Diamant

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Abstract

Diamant B *Observations on anaphylactic and compound 48/80-induced histamine release from guinea pig and rat lung tissue in vitro* Acta physiol scand 1962 55 11—25 — The effect of oxygen lack on anaphylactic histamine release from sensitized guinea pig and rat lung tissue *in vitro* has been investigated and found to consist of pronounced inhibition provided that the incubation medium (phosphate buffer) was devoid of glucose In the presence of glucose this inhibition was overcome progressively with rising concentrations of glucose Under oxygen the anaphylactic histamine release from guinea pig lung tissue was also enhanced by the presence of glucose By comparing anaphylactic histamine release from guinea pig lung tissue and Compound 48/80 induced histamine release from rat lung tissue in glucose containing phosphate buffer and Tyrode solution respectively it has been demonstrated that pH changes produced by gassing the Tyrode solution with oxygen and nitrogen have obscured the true effect of nitrogenation in earlier investigations performed in glucose containing Tyrode solution

It is generally agreed that anaphylactic histamine release *in vitro* from guinea pig lung tissue involves energy requiring reactions This conclusion is based on the findings that oxygen lack (Parrot 1942 Mongar and Schild 1957 Chakravarty 1960 Moussatché and Provoust Danon 1960 Yamasaki Muraoka and Endo 1960) and inhibitors of oxidative phosphorylation (Mongar and Schild 1957 Moussatché and Provoust Danon 1958) depress anaphylactic release of histamine These findings have been interpreted as a

dependence on intact oxidative metabolic pathways for the energy production furnishing the anaphylactic histamine releasing reaction in the guinea pig

In contrast both anaphylactic and Compound 48/80 induced histamine release *in vitro* from rat lung tissue was reported to be uninfluenced by oxygen lack (CHAKRAVARTY 1960) This suggested that the energy production could be furnished anaerobically in this species In support of this hypothesis CHAKRAVARTY (1959) put forward the observation of Diamant i e that as long as the incubation medium contained glucose the histamine release elicited from rat lung tissue by extracts of *Ascaris suis* appeared despite oxygen lack In the absence of glucose on the other hand the histamine release was markedly inhibited (DIAMANT 1961) In the rat, glucose and oxygen lack were both found to have a similar effect on histamine release as well as on mast cell degranulation when elicited by the polymer amine Compound 48/80 (DIAMANT and UVNÄS 1961)

In the present investigation, the anaphylactic histamine release *in vitro* from rat and guinea pig lung tissue has been studied with respect to the effect of glucose under aerobic and anaerobic conditions Furthermore in order to evaluate the varying results reported in earlier investigations as to the effect of oxygen lack anaphylactic histamine release from guinea pig lung tissue has been compared with that elicited by Compound 48/80 from rat lung tissue in different incubation media

Material and Methods

Guinea pigs (male and female 250—350 g) and rats (male and female 200—300 g) were sensitized by injection of crystallized egg albumin Guinea pigs were injected subcutaneously with 0 1 g and intraperitoneally with 0 1 g followed 3 days later by a s c injection of 0 1 g Rats were injected s c with 0 1 g of egg albumin together with 1 ml of *H pertussis* vaccine (2×10^{10} bacilli per ml) followed 3 days later by another s c injection of 0 1 g of egg albumin The sensitized guinea pig lungs were used for *in vitro* anaphylactic reactions 3—25 weeks after the first sensitizing dose and the sensitized rat lungs 3—8 weeks after it

The lungs of the sensitized animals were treated and incubated as described in detail earlier (DIAMANT 1961) with modifications according to DIAMANT and UVNÄS (1961) In the experiments where the effect of Compound 48/80 was investigated non sensitized rat lungs were used and treated by the same method

Two different solutions were used for preparation and incubation of the lung tissue (1) A solution (denoted in the following as phosphate buffer) containing NaCl (154 mM) KCl (2 7 mM) and $CaCl_2$ (0 9 mM) buffered with 10 % v/v Sörensen phosphate buffer (67 mM) The initial pH of the phosphate buffer ranged from 7 1 to 7 2 in the single experiments (2) Tyrode solution containing NaCl (137 mM) KCl (2 7 mM) $CaCl_2$ (1 8 mM) $MgCl_2$ $6H_2O$ (1 0 mM) $NaHCO_3$ (11 9 mM) and NaH_2PO_4 H_2O (0 4 mM) The initial pH of the Tyrode solution ranged from 7 7 to 8 3 in the single experiments Neither the phosphate buffer nor the Tyrode solution contained glucose i e during preparation the lung tissue was treated with a glucose free solution in all experiments The interval between sacrificing the animals and the incubation of the lung tissue amounted to 1 1/2—3 hours

Table I Spontaneous histamine release from rat and guinea pig lung tissue in phosphate buffer under the influence of oxygen nitrogen and glucose (5 6 mM) Histamine release computed in of total histamine content mean and standard error of the mean Bracketed figures denote the number of experiments

Species	Spontaneous histamine release			
	O + glucose	N_2 + glucose	O	N
Rat	8 0 ± 0 69 (10)	6 6 ± 0 40 (23)	6 7 ± 0 53 (13)	7 0 ± 0 45 (28)
Guinea p g	1 1 ± 0 14 (5)	1 3 ± 0 09 (6)	1 0 ± 0 23 (4)	1 0 ± 0 13 (6)

In some experiments in which the effect of phosphate buffer and Tyrode solution on histamine release was investigated the lung tissue was cut into coarse pieces in physiologic sodium chloride solution After thorough mixing it was divided into two parts by weighing one part was then treated and incubated in phosphate buffer and the other in Tyrode solution

The gaseous phases used during incubation usually consisted of commercially pure oxygen or nitrogen In some experiments however gas mixtures composed of oxygen or nitrogen (93 5 %) together with CO_2 (6 5 %) were used

The lung tissue was exposed to crystallized egg albumin (1 mg/ml) or Compound 48/80 (35 μg/ml) for 20 min in all experiments After withdrawal of the incubation fluids their pH was determined electrometrically All concentrations of substances given in the text refer to the final concentration in the incubation fluid

The remaining histamine of the lung tissue after incubation was extracted by heating to 100 C for 10 min This was done in a slightly acid medium (pH 3—4) by addition of N HCl in all experiments where the lung tissue was extracted in Tyrode solution (in order to avoid breakdown of histamine at alkaline pH) as well as when guinea pig lung tissue was extracted in phosphate buffer (in order to inactivate substances interfering with the histamine assay on the isolated guinea pig ileum) In the experiments where the effect of phosphate buffer and Tyrode solution was investigated simultaneously all lung samples were extracted with a phosphate buffer All acidified extraction samples were neutralized before histamine assay

Histamine was assayed on atropinized guinea pig ileum At least 4 single contractions of each sample were compared with a known histamine dihydrochloride solution In random samples mepyramin completely inhibited the contractions

Unless otherwise stated the histamine release was computed in % of the total histamine content of the lung tissue The amount of histamine found in controls without antigen or Compound 48/80 (denoted as spontaneous histamine release) was deducted from all values given in the text

Results

Spontaneous histamine release from guinea pig and rat lung tissue

The spontaneous histamine release from guinea pig and rat lung tissue in phosphate buffer under the influence of oxygen nitrogen and glucose is shown in Table I No significant difference between the histamine release under the various metabolic conditions was observed within the same species A significant difference was on the contrary noted in a comparison between guinea pig

dependence on intact oxidative metabolic pathways for the energy production furnishing the anaphylactic histamine releasing reaction in the guinea pig

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Two different solutions were used for preparation and incubation of the lung tissue (1) A solution (denoted in the following as phosphate buffer) containing NaCl (154 mM) KCl (2 7 mM) and $CaCl_2$ (0 9 mM) buffered with 10 % v/v Sörensen phosphate buffer (67 mM) The initial pH of the phosphate buffer ranged from 7 1 to 7 2 in the single experiments (2) Tyrode solution containing NaCl (137 mM) KCl (2 7 mM) $CaCl_2$ (1.8 mM) $MgCl_2$ $6H_2O$ (1 0 mM) $NaHCO_3$ (11 9 mM) and NaH_2PO_4 H_2O (0 4 mM) The initial pH of the Tyrode solution ranged from 7 7 to 8 3 in the single experiments Neither the phosphate buffer nor the Tyrode solution contained glucose i e during preparation the lung tissue was treated with a glucose free solution in all experiments. The interval between sacrificing the animals and the incubation of the lung tissue amounted to 1 1/2—3 hours

Table II Anaphylactic histamine release from guinea pig lung tissue in phosphate buffer under the influence of oxygen nitrogen and glucose (5 6 mM) Histamine release computed in ' of total histamine content Spontaneous release deducted from all values Bracketed figures denote the pH of the phosphate buffer after incubation

Experiment no	Initial pH of phosphate buffer	Histamine release			
		O + glucose	O	N + glucose	N
1	7 1	38 7 (6 8)	27 0 (7 0)	20 2 (6 8)	0 0 (7 0)
2	7 2	29 9 (6 9)	19 3 (7 1)	23 4 (6 9)	0 3 (7 0)
3	7 2	34 5 (6 9)	25 1 (7 0)	26 0 (6 8)	2 6 (7 0)
4	7 2	36 1 (6 9)	29 7 (7 1)	27 2 (6 8)	2 7 (7 0)
5	7 2	38 1 (6 9)	23 8 (7 0)	34 5 (6 8)	7 9 (7 0)
6	7 2	20 9 (6 9)	11 6 (7 2)	16 2 (6 9)	0 3 (7 1)
7	7 ?	22 6 (6 9)	11 4 (7 1)	16 3 (6 9)	0 4 (7 1)
Mean and standard error of mean		31 5 ± 2 8	21 1 ± 2 8	23 4 ± 2 5	2 0 ± 1 1
Significance of difference of mean from O + glucose (*t* test)			P = 0 05—0 01	P = 0 05—0 01	P = < 0 001

Table III Effect of glucose (5 6 mM) on anaphylactic histamine release from rat lung tissue in phosphate buffer under nitrogen Histamine release computed in of total histamine content Spontaneous release deducted from all values Bracketed figures denote the pH of the phosphate buffer after incubation

Experiment no	Initial pH of phosphate buffer	Histamine release	
		N + glucose	N
1	7 2	30 4 (7 0)	< 4 0 (7 1)
2	7 2	31 5 (7 0)	0 0 (7 2)
3	7 2	14 0 (6 9)	0 3 (7 0)
4	7 2	29 2 (6 9)	1 1 (7 0)
5	7.2	20 3 (6 9)	0 0 (7 1)
	Mean	25 1	< 1 1

pig lung tissue are given in Table II Histamine release was found to be optimal under oxygen in the presence of 5 6 mM glucose amounting on the average to 32 % of the total histamine content Under oxygen in the absence of glucose 21 % was released The histamine release under nitrogen in the absence of glucose averaged 2 % of the total histamine content it was raised to 23 % by addition of 5 6 mM glucose Table II shows in addition the significance of the differences from the optimal histamine release (i e with oxygen and glucose) The pH of the phosphate buffer before and after incubation with the lung

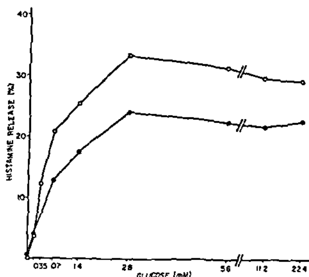


Fig 2 Effect of various concentrations of glucose on anaphylactic histamine release from rat (— — —) and guinea pig (— — —) lung tissue in nitrogenated phosphate buffer Histamine release computed in % of total histamine content Spontaneous histamine release deducted from all values

tissue under the various experimental conditions is also recorded in Table II In every experiment the decrease in pH of the phosphate buffer after incubation was somewhat greater in the presence of glucose Thus the pH was usually 0 2 units lower than that of phosphate buffer incubated in the absence of glucose

The effect of 5 6 mM glucose on the histamine release from sensitized rat lung tissue under oxygen lack is shown in Table III (5 separate experiments) The average histamine release amounted to about 1 % of the total histamine content in the absence of glucose, as compared to 25 % in the presence of glucose The fall in pH of the phosphate buffer after incubation was slightly greater (0 1—0 2 units) in the presence of glucose than in its absence

The effect of different concentrations of glucose on the anaphylactic histamine release from guinea pig and rat lung tissue in phosphate buffer and under oxygen lack is shown in Fig 2 The histamine release increased progressively with rising glucose concentration up to 2 8 mM (0 5 mg/ml) in both species An increase in glucose concentration above 2 8 mM had no greater effect on histamine release *Judging by the curves in Fig 2 0 25 mM* (0 045 mg/ml) is about the lowest concentration that would produce an appreciable increase in histamine release in both species as compared to that in nitrogenated buffer without addition of glucose

Effect of oxygen nitrogen carbon dioxide and glucose on anaphylactic histamine release from guinea pig lung tissue and on Compound 48/80 induced histamine release from rat lung tissue in phosphate buffer and Tyrode solution

There is a considerable discrepancy between the data given by various authors on the inhibition of anaphylactic histamine release under oxygen lack

Table II Effect of oxygen lack on anaphylactic histamine release from sensitized lung tissue as reported in earlier investigations

Investigator	Species	Incubation medium	Glucose present in incubation medium	Histamine release under oxygen lack
PARROT (1942)	Guinea pig	Tyrode solution	?	Inhibition (no values given)
MONGAR and SCHILD (1957)	Guinea pig	Tyrode solution	5 6 mM	37 (range 6—55 of histamine release in aerated medium
CHAKRAVARTY (1960)	Guinea pig	Tyrode solution	*5 6 mM	14—29 of histamine release in oxygenated medium
MOUSSATCHÉ and PROVOUST DANON (1960)	Guinea pig	Ringer Barron solution	0	0 of histamine release in oxygenated medium
YAMASAKI MURAOKA and ENDO (1960)	Guinea pig	Krebs-Ringer solution	0	0 of total histamine content released
CHAKRAVARTY (1960)	Rat	Tyrode solution	*5 6 mM	No inhibition

* Personal communication.

Earlier reports on the effect of oxygen lack on the histamine release from sensitized guinea pig and rat lung tissue are summarized in Table IV

It can be seen that anaphylactic histamine release from guinea pig lung tissue was incompletely and variably inhibited by oxygen lack in a medium containing glucose (Tyrode solution) This is in contrast to the complete inhibition noted in incubation media devoid of glucose (Krebs Ringer and Ringer Barron solution) It is also evident that the histamine release from the sensitized lung tissue of the rat — unlike that of the guinea pig — was found to be uninhibited in Tyrode solution despite oxygen lack CHAKRAVARTY (1960) *also reported that Compound 48/80-induced histamine* release from rat lung tissue in Tyrode solution was uninfluenced by oxygen lack

I therefore considered it of interest to investigate whether the varying effect of anoxia on histamine release in Tyrode solution observed in earlier studies was due to the glucose present or whether some other factor might be involved Consequently it was decided to compare the histamine release in phosphate buffer and in Tyrode solution in order to evaluate the differences produced by the incubation media

The effect of 5 6 mM glucose on the anaphylactic histamine release from *guinea pig lung tissue under oxygen and nitrogen in phosphate buffer* and in Tyrode solution is shown in Fig 3 In oxygenated glucose containing

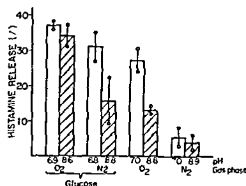


Fig 3 Effect of oxygen nitrogen and glucose (5 6 mM) on anaphylactic histamine release from guinea pig lung tissue in phosphate buffer (unshaded blocks) and Tyrode solution (shaded blocks) Mean values of two experiments are shown open and filled circles represent the individual values in each experiment The final pH of the incubation medium is given below each block Initial pH of incubation media phosphate buffer 7 2 Tyrode solution 8.2 Histamine release computed in of total histamine content Spontaneous release deducted from all values

phosphate buffer the release averaged about 37 % of the total histamine content against about 34 % in oxygenated glucose containing Tyrode solution In correspondingly nitrogenated glucose containing incubation media the histamine release averaged 31 % and 15 %, respectively In oxygenated glucose free phosphate buffer, 27 % was released against 13 % in oxygenated glucose free Tyrode solution, whereas 6 % and 4 % respectively was released in correspondingly nitrogenated glucose free media It was further noted that both oxygenation and nitrogenation of the Tyrode solution during incubation raised the final pH to 8 6—8 9

It is evident that the difference between the histamine release in the two incubation media was negligible under oxygen in the presence of glucose In nitrogenated glucose containing Tyrode solution however the histamine release markedly decreased as compared to that in phosphate buffer under equal experimental conditions This difference is further shown in Table V, which gives the individual values in 5 experiments on the histamine release from sensitized guinea pig lung tissue under oxygen lack in glucose containing Tyrode solution and phosphate buffer respectively together with the pH of the incubation media before and after incubation It is seen that the histamine release in Tyrode solution ranged from 10 % to 63 % of that in phosphate buffer in the various experiments This is in contrast to the small variations within single experiments (exp 4 and 5 Table V) The pH of the phosphate buffer which was initially 7 2 in all experiments fell during incubation to 6 8—6 9 In Tyrode solution on the contrary the pH rose to 8 8 in every experiment despite an initial range of 7 7—8 3

It is obvious from the reports of Mongar and Schild (1958) and Chakravarty (1960) that anaphylactic histamine release from guinea pig lung tissue is not optimal at pH 8 8 These workers used Tyrode solution as incubation medium when studying the influence of oxygen lack on histamine release Consequently changes in the pH of the solution towards the alkaline side due to the experimental conditions might have obscured the true effect of oxygen lack in these investigations

Table 1 Anaphylactic histamine release from guinea pig lung tissue under nitrogen in phosphate buffer and Tyrode solution in the presence of glucose (5 6 mM) Histamine release computed in of total histamine content Spontaneous release deducted from all values Bracketed figures de note the pH of the solutions after incubation Initial pH of incubation media phosphate buffer 7 2 Tyrode solution 7 7—8 3 Variations in histamine release in double and triple tests are shown (ex periments 4 and 5) $\frac{B}{A} \times$ *100 denotes histamine release in Tyrode solution computed in of that in phosphate buffer*

Experiment no	Histamine release				
	Phosphate buffer (A)		Tyrode solution (B)		$\frac{B}{A} \times 100$
1	16.3 (6 9)		4 1 (8 8)		25
2	34 5 (6 8)		21 6 (8.8)		63
3	27 2 (6 8)		9 1 (8.8)		34
4	27 1 (6 9)	26 5 (6 9) 27 6 (6 9)	2 8 (8 8)	1 4 (8 8) 4 3 (8 8)	10
5	19 6 (6 8)	20 0 (6 8) 20 1 (6 9) 18 9 (6 8)	7 2 (8 8)	7 4 (8 8) 6 5 (8 8) 7 6 (8 8)	45

In order to test this hypothesis the pH changes of phosphate buffer and Tyrode solution (initial pH 7 2 and 7 8 respectively) were first studied in the absence of lung tissue after exposure to oxygen and nitrogen with and without CO_2 (6 5 %) for 30 min at 37 C After exposure to oxygen and nitrogen without CO_2 the final pH was 7 3 in phosphate buffer and 9 1 in Tyrode solu tion In the presence of CO_2 the pH fell to 6 5 in phosphate buffer and to 7 1 in Tyrode solution both with oxygen and nitrogen In the absence of lung tis- sue addition of 5 6 mM glucose did not influence the final pH

In view of these pH changes rat and guinea pig lung tissue respectively (pooled from 3—5 animals) was divided into two parts One part was prepared and incubated in Tyrode solution and the other in phosphate buffer Nitrogen alone or a mixture of nitrogen (93 5 %) and CO_2 (6 5 %) was used during incu bation in order to achieve anoxic experimental conditions It is apparent from Figs 4 and 5 that in glucose containing (5 6 mM) Tyrode solution both ana phylactic histamine release from guinea pig lung tissue (Fig 4) and Compound 48/80-induced histamine release from rat lung tissue (Fig 5) were markedly en hanced by CO_2 under oxygen lack Without CO_2 the final pH of the Tyrode solu- tion was 8 7—8 9 in the presence of CO_2 it fell to 7 5—7 6 In glucose con taining (5 6 mM) phosphate buffer on the other hand CO_2 instead induced a decrease in histamine release as compared to that under nitrogen without CO_2 In both experiments the final pH of the phosphate buffer was 6 9 without CO_2 whereas it fell to 6 6 in the presence of CO It is further evident that in both

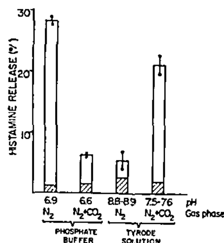


Fig 4

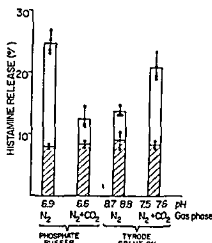


Fig 5

Fig 4 Effect of nitrogen and of nitrogen (93 5) + CO (6 5 %) on anaphylactic histamine release from guinea pig lung tissue in glucose-containing (5 6 mM) phosphate buffer and Tyrode solution Mean values of double tests in one experiment are shown Unshaded blocks antigen induced histamine release shaded blocks spontaneous histamine release The final pH of the incubation medium is given below each block Initial pH of incubation media phosphate buffer 7 2 Tyrode solution 7 7 Histamine release computed in of total histamine content

Fig 5 Effect of nitrogen and of nitrogen (93 5) + CO_2 (6 5) on Compound 48/80-induced histamine release from rat lung tissue in glucose containing (5 6 mM) phosphate buffer and Tyrode solution Open and filled circles represent individual values in two experiments Unshaded blocks Compound 48/80-induced histamine release (mean value of double tests) Shaded blocks spontaneous histamine release (mean value of single tests) The final pH of the incubation medium is given below each block Initial pH of incubation media phosphate buffer 7 2 Tyrode solution 7 8 Histamine release computed in of total histamine content

reactions the histamine release under nitrogen in the absence of CO_2 was markedly inhibited in Tyrode solution as compared with that in phosphate buffer

The effect of CO_2 (6 5 %) on the histamine release in oxygenated and nitrogenated Tyrode solution *in the presence of glucose* (5 6 mM) is shown in Fig 6 (antigen and guinea pig lung tissue) and Fig 7 (Compound 48/80 and rat lung tissue) Under nitrogen in the presence of CO_2 (pH 7 7) about 25 % of the total histamine was released from the lung tissue by omitting CO_2 (pH 8 8) it decreased to about 6 % in both experiments Under oxygen in the presence of CO_2 (pH 7 6) about 31 % of the total histamine was released in both experiments, without CO_2 (pH 8 6) the histamine release decreased to about 8 % in the rat but was almost uninfluenced in the guinea pig (about 27 %)

Discussion

The present investigation has shown that under oxygen lack the anaphylactic histamine release from both guinea pig and rat lung tissue depends on the glucose present in the incubation medium (phosphate buffer) This can be ex

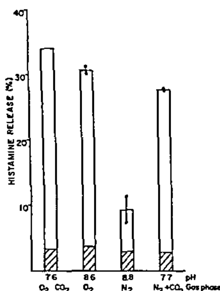


Fig 6

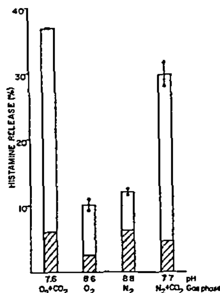


Fig 7

Fig 6 Effect of oxygen and nitrogen with and without CO_2 (6 5) on anaphylactic histamine release from guinea pig lung tissue in glucose containing (5 6 mM) Tyrode solution Filled circles represent individual values of double tests in one experiment Unshaded blocks antigen induced histamine release shaded blocks spontaneous histamine release The final pH of the Tyrode solution is given below each block. Initial pH of Tyrode solution 7 8 Histamine release computed in of total histamine content

Fig 7 Effect of oxygen and nitrogen with and without CO (6 5) on Compound 48 80-induced histamine release from rat lung tissue in glucose-containing (5 6 mM) Tyrode solution. Filled circles represent individual values of double tests in one experiment Unshaded blocks Compound 48/80-induced histamine release shaded blocks spontaneous histamine release The final pH of the Tyrode solution is given below each block Initial pH of Tyrode solution 7 9 Histamine release computed in of total histamine content.

plained on the assumption that anaphylactic histamine release involves an energy requiring reaction The results have shown that histamine release can occur without aerobic metabolic reactions indicating that under oxygen lack glycolysis is able to furnish energy This is in good agreement with earlier findings on the histamine release from rat lung tissue when induced by Ascaris extract (Diamant 1961) and Compound 48 80 (Diamant and Uvnäs 1961)

Under oxygen the anaphylactic histamine release from guinea pig lung tissue is also enhanced by glucose (5 6 mM) although the increase is less pronounced than under oxygen lack A possible explanation of this effect of glucose in the presence of oxygen is that the store of energy yielding substances is diminished by the experimental procedures but is refilled by the addition of glucose

The histamine releasing reactions initiated in anaphylaxis as well as by the synthetic and biologically occurring histamine releasers so far tested thus might involve an energy requiring step Whether this step is com

mon to all these histamine releasing systems is so far unknown In favour of such a hypothesis is the good agreement in the dose effect relations between the anoxic histamine release elicited by the different agents and the glucose concentration Furthermore, the same concentration of phlorizin was found to cause about 50 % inhibition of the anaphylactic histamine release from guinea pig lung tissue as well as of Compound 48/80 induced histamine release from rat lung tissue in nitrogenated and glucose containing phosphate buffer (DIAMANT 1960) On the other hand certain differences have been noted Firstly there is a quantitative difference between anaphylactic histamine release from guinea pig lung tissue under oxygen and nitrogen respectively, in the presence of 5 6 mM glucose This is in contrast to the histamine release from rat lung tissue induced by Compound 48/80 under similar experimental conditions where no statistical difference was present (DIAMANT and UVNAS 1961) Secondly, a qualitative difference was observed between anaphylactic histamine release from guinea pig lung tissue and Compound 48/80 induced histamine release from rat lung tissue under oxygen, in the former reaction, glucose could be replaced by sodium succinate whereas in the latter sodium succinate had no effect (DIAMANT 1960)

It is not yet known whether these findings are due to species differences (as suggested by CHAKRAVARTY 1960), or to differences in the reactions involved in anaphylaxis and Compound 48/80 induced histamine release (as suggested by MONGAR and SCHILD 1957 and MOUSSATCHÉ and PROVOUST DANON 1957 on the basis of guinea pig experiments) A possible way of solving this problem would be to perform comparative studies on anaphylactic histamine release from rat lung tissue Experiments of this nature are now in progress

When comparing the histamine release induced by antigen from sensitized guinea pig lung tissue and by Compound 48/80 from rat lung tissue in phosphate buffer and Tyrode solution respectively, it was found that oxygenation as well as nitrogenation raised the pH of the Tyrode solution to 8 6—8 9 in the presence of lung tissue Gassing the Tyrode solution in the absence of lung tissue shifted the pH even further to the alkaline side (pH 9 1) The lung tissue itself evidently prevents the pH from rising to the expected value of 9 1 When *buffer solution was similarly gassed the pH rose from the initial 7 2 only* to 7 3 in the absence of lung tissue In the presence of lung tissue pH values from 6 8 to 7 2 were recorded It is notable that the range of the values obtained (in the presence of lung tissue) and those expected (in the absence of lung tissue) was of the same order in the two incubation media used

An additional finding was that in the presence of 5 6 mM glucose the final pH values were 0 1—0 2 units lower than in the absence of glucose provided that lung tissue was present during incubation (Table II and III Fig 3) This decrease in pH produced by the presence of glucose was noted under both oxygen and nitrogen and is most likely due to the formation of lactic acid through the breakdown of glucose in the lung tissue

In the presence of 5 6 mM glucose the anaphylactic histamine release from guinea pig lung tissue as well as the histamine release induced by Compound 48/80 from rat lung tissue was found to be markedly inhibited in nitrogenated Tyrode solution as compared to the values in phosphate buffer under identical conditions (Fig 3 4 and 5 Table V) This inhibition of histamine release in glucose containing Tyrode solution — which in the case of anaphylactic release from guinea pig lung tissue ranged from 37 to 90 % in 5 exp — is suggested to be due to the induced alkaline pH This is evidenced by the finding that gassing with a mixture of nitrogen (93 5 %) and CO_2 (6 5 %) instead of nitrogen alone had a markedly activating effect on both histamine releasing reactions with a concurrent fall in pH to a more physiological level (Fig 4 5 6 and 7)

Another conceivable explanation is that this activation of histamine release is due to the CO *per se* It was however found that when the same mixture of nitrogen and CO_2 was used in glucose containing phosphate buffer the histamine release was markedly inhibited as compared to that in glucose-containing phosphate buffer gassed with nitrogen alone (Fig 4 and 5) This inhibitory effect of CO_2 on the histamine release in glucose containing phosphate buffer might be ascribed to the fall in pH to 6 6 In both histamine releasing reactions the values recorded in glucose containing phosphate buffer gassed with nitrogen and CO (pH 6 6) were of the same order as those in glucose containing Tyrode solution gassed with nitrogen alone (pH $\approx$ 8 8)

The effect of CO_2 (6 5 %) on the pH and on the histamine release was further studied in nitrogenated and oxygenated glucose containing Tyrode solution as shown in Fig 6 (antigen and guinea pig lung tissue) and Fig 7 (Compound 48/80 and rat lung tissue) In both experiments nitrogen alone induced a pH of 8 8 whereas in the presence of CO_2 the pH decreased to 7 7 Under oxygen alone the final pH was 8 6 with a fall to 7 6 in the presence of CO_2 In both histamine releasing reactions nitrogen alone produced an inhibition of histamine release as compared to the values recorded in the presence of CO Oxygen alone had an inappreciable effect on the anaphylactic histamine release from guinea pig lung tissue when compared to the values recorded in the presence of CO This is in contrast to the effect of oxygen alone on Compound 48/80 induced histamine release from rat lung tissue which was inhibited to the same degree as under nitrogen alone

The discrepancies in earlier reports on the effect of anoxia on histamine releasing reactions with the use of Tyrode solution were tentatively ascribed to the varying presence of glucose in the incubation medium (DIAMANT 1961) This explanation cannot however be relevant since personal communications from CHAKRAVARTY (1961) and SCHILD (1961) disclosed that the Tyrode solution in their experiments always contained 5 6 mM glucose The present report demonstrates instead why a difference is present between anaphylactic histamine release from guinea pig lung tissue and Compound 48/80 induced histamine release from rat lung tissue when the effects of oxygenation and nitrogenation

are studied in glucose containing Tyrode solution Apparently Compound 48/80 induced histamine release from rat lung tissue under oxygen (pH 8 6) is inhibited to the same degree as under nitrogen (pH 8 8) Anaphylactic histamine release from guinea pig lung tissue under oxygen on the other hand is only *slightly affected (if at all) at pH 8 6 whereas under nitrogen (pH 8 8)* the release is inhibited to numerically the same degree as in rat lung tissue

The reason for this difference between the histamine release in the two systems tested under oxygen at pH 8 6 is hitherto unknown Further investigations are needed to determine whether it is due to a species difference, or to a difference between the mechanisms of anaphylactic and Compound 48/80 induced histamine release Whatever the reason may be this difference has obscured the true effect of nitrogenation in earlier investigations performed in glucose containing Tyrode solution

The present report shows that when an incubation medium is used (phosphate buffer) of which the pH is unaffected by oxygenation or nitrogenation the anoxic experimental conditions induced by nitrogenation cause a pronounced inhibition of anaphylactic histamine release from both guinea pig and rat lung tissue Moreover this inhibition can be counteracted by the presence of glucose in the incubation medium

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Acta physiol scand 1962 55 26—34

From the Chemical Central Laboratory Sabbatsberg Hospital Stockholm Sweden and the Department of Experimental Surgery Thoracic Clinic Karolinska Institute Stockholm Sweden

On the Reversible Extraction of Bromsulfalein (BSP) in the Liver

By

Bo Norberg, Åke Senning and Goran William Olsson

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Abstract

Norberg B Å Senning and G William Olsson *On the reversible extraction of bromsulfalein (BSP) in the liver* Acta physiol scand 1962 *55* 26—34 — Through a charging of the liver of dogs with radioactive bromsulfalein and subsequent challenge experiments with single doses of inactive bromsulfalein it is shown that the absorption of bromsulfalein from the blood is an apparently reversible process The amount of bromsulfalein given off by the liver to the blood constitutes a considerable fraction of absorbed and transmitted BSP This is calculated with different methods from the analytical values for blood samples from the portal vein and the isolated branches of the hepatic veins

The use of bromsulfalein BSP for the testing of the hepatic function is an important step in the diagnosis of cirrhosis and similar conditions The interpretation however is by no means quite clear In the retention test according to Rosenthal and White (1925) the same answer may be obtained in different ways which has led MacDonald (1939) to introduce a so-called clearance test which may suitably be read and reported as the first half life for the concentration in the plasma after a single injection The halving time as a criterion of the functional state of the liver presupposes that the extraction in the liver is proportional to the plasma concentration of BSP *i e* that the percentual extraction (the extraction factor) is constant In clinical clearance measurements one does certainly frequently get a logarithmically decreasing BSP concentration in the peripheral blood But in measurements on either

side of the liver VETTER *et al* (1956) adduces a constantly decreasing extraction factor and in experiments on animals we have made the same observation after single doses of BSP (ANDERSEN, NORBERG and SENNING 1958). In the last mentioned work the possibility that a certain proportion of the BSP of the liver-cells return to the blood during the passage through the liver is adduced as an attempt at explanation. The apparent extraction factor is then the equilibrium value for two processes. The present work is an attempt to prove the above hypothesis.

Methods

Animal Experiments

Experiments have been carried out on dogs anesthetized with Nembutal. In order to eliminate the big operative trauma entailed in the earlier method for the isolation of the flow of hepatic blood the following technique has been used. About one month before the BSP experiment the abdomen of the animal was opened with a mid line incision, a ligature was passed around the vena cava inferior just distal to the hepatic veins and the ends of the ligature were drawn through a polyethylene catheter which was pushed against v. cava so that the latter was strangulated to about one third its original diameter. The catheter was then fixed by knotting the ligature and subcutaneous positioning. After about 14 days the catheter was exposed and the ligature drawn tight to occlude the cava totally. After a week a vena-cava angiography was made to ensure that v. cava was closed. It was then also possible to observe that collateral vessels had developed. The suprahepatic part of v. cava thus carried only blood from the liver.

When the BSP-experiment was to be carried out the animal was anesthetized and the portal vein was exposed via a small muscle splitting incision and a fine polyethylene catheter inserted. The dog was heparinized with approximately 4 mg heparin/kg body weight. From the external jugular vein on the right side a stout balloon catheter was pushed down into v. cava inferior so that when the ballon was inflated supra hepatically all the hepatic blood ran through the catheter and back to the external jugular vein on the left side. In some experiments the flow of blood through the liver was measured by determining the time needed for the collection of 100 g blood in a rubber balloon placed on one pan of a balance. Blood samples were taken through a thin catheter in the balloon catheter and from the catheter in the portal vein.

In order to try to show the yield of BSP also from the liver to the venous blood the liver was first charged with radioactive BSP (^{35}S-marked BSP supplied by the Radiochemical Centre, Amersham) with a single dose of about 2—16 mg/kg body weight. After this a dose of inactive BSP was given and BSP and radioactivity were followed in the hepatic venous blood and in portal blood.

Analytic Methods

Bromsulfalein in serum was determined according to SELIGSON, MARINO and DODSON (1957).

Radioactivity was determined first according to DOHLMAN (1957) through wet digestion with nitric acid and hydrogen peroxide in the presence of sulfuric acid. Aliquots of the final water solution were evaporated on lead planchettes and counted in a windowless flow counter (Nuclear Measurements Co. Proportional Counter Con-

Table I Changes in specific activity in portal and hepatic blood during bromsulfalein charging following loading of the liver with radioactive bromsulfalein. Minimum radioactive bromsulfalein to account for increase in specific activity indicated in last two columns

Exp no	Charge			Time from injection	Spec activity cps/mg BSP		Minimum added mg ^{35}S-BSP per mg total	
	No	mg BSP/kg		min	Portal	Hepatic	Portal	Hepatic
600 406	I	5	inactive	—	—	—	—	—
	II	3	S 35	—	—	—	—	—
	III	5	inactive	5	1 680	1 900	—	—
				8	2 300	2 750	0 06	0 08
				14	2 690	3 520	0 10	0 16
600 428	I	2 2	S 35	—	—	—	—	—
	II	30 6	inactive	5	40?	450	—	—
				8	511	588	0 01	0 01
				14	685	810	0 03	0 04
600 819	I	6 1	S 35	—	—	—	—	—
	II	9 7	inactive	1	2 770	2 650	—	—
				7	8 080	7 600	0 59	0 48
				10	11 000	9 0 0	0 79	0 62
	III	4 4	inactive	1	1 570	1 740	—	—
				7	3 9?0	4 2?0	0 23	0 24
				10	4 730	4 730	0 30	0.29
600 826	I	8 9	S-35	—	—	—	—	—
	II	18 6	inactive	3	1 440	2 460	—	—
				9	? 700	4 010	0 12	0 15
				15	4 080	5 450	0 25	0 ??
				20	4 830	6 240	0 33	0 36
	III	9 4	inactive	5	2 030	2 090	—	—
				8	2 510	2 9?0	0 04	0 08
				14	3 450	4 220	0 13	0 ?1
				25	3 700	5 100	0 16	0 ?9

verter PCC 14 This arrangement gave about 13 counts per second cps per nanocurie (10 C Although the biggest aliquot was 0 15 ml serum corresponding to a dry weight of only 10 mg a noticeable self absorption was obtained in a considerable number of cases As soon as there was a possibility of carrying out liquid scintillation counting the determinations were instead performed according to JEFFAY OLUBAJO and JEWELL (1960) when no significant self absorption was observed With an Ekco Electronics Ltd Scintillation Counter N 664 A we obtained on an average 29 cps per nanocurie

Hematocrit was measured according to NORBERG and WARVENIUS (19?)

The plasma volume was determined after the BSP injections or in the majority of experiments simultaneously with the second dose of inactive BSP with the help of ^{131}I albumin (Amersham

Table II Experiments as in Table I with another bromsulfalein preparation
Liquid scintillation counting

Exp no	Charge		Time from injection	Spec activity cps/mg BSP		Minimum fraction of BSP as $B^{35}SP$	
	No	mg BSP/kg	min.	Portal	Hepatic	Portal	Hepatic
601 097	I	4.5 S 35	—	—	—	—	—
	II	17 1 inactive	3	9 450	14 350	—	—
			6	12,250	20 350	0 02	0 05
			14	—	29 100	—	0 13
			19	16 400	31 400	0 06	0 15
601,209	I	15 3 S-35	—	—	—	—	—
	II	10 5 inactive	3	27 600	29 500	—	—
			9	29 900	34,200	0 02	0 04
			12	33,200	41 500	0 05	0 10
			18	44 100	45 800	0 14	0 14
	III	3 7 inactive	3	28 000	28 600	—	—
			9	30 600	33 900	0 02	0 05
			18	41 700	36,200	0 11	0 07
601,220	I	16 7 S-35	—	—	—	—	—
	II	27 6 inactive	3	7 350	12 300	—	—
			10	13 600	19 800	0 05	0 06
			16	16 600	25,200	0 08	0 11
			24	*23 000*	*32 000*	*0 14*	*0 17*
	III	18 3 inactive	3	13 400	20,200	—	—
			12	15 600	22,400	0 02	0 03
			15	17 700	26,200	0 04	0 05
			25	22 400	24 000	0 08	0 03

Results

After charging the liver with radioactive bromsulfalein to provoke the release of labelled bromsulfalein from the liver single injections of inactive bromsulfalein referred to as challenge experiments were carried out Two different preparations of radioactive bromsulfalein were used The first series comprises six challenge experiments on 4 dogs and is presented in abridged form in Table I Seven challenge experiments were carried out with the other preparation 5 of these being presented in Table II The tables give the dose of bromsulfalein in mg per kg body weight for each charging experiment as well as the specific activities calculated from the analytical values in impulses per second per mg bromsulfalein in peripheral blood represented by the blood samples from the portal vein and in the blood from the isolated hepatic veins On the assumption that the specific activity of the bromsulfalein of the liver at the beginning of the challenge experiment is the same as in the radioactive preparation used (for the first series 10 400 cps/mg for the day to which all

Table III Excess output of radioactivity in hepatic blood calculated from mean liver plasma flow and extraction factor Excess output in mg sulfobromophtalein/min based on specific activity of 116 000 cps/mg in the liver

A Exp 601 220 II liver plasma flow 187 ml/min extr factor 18

B Exp 610 317 II plasma flow 71 ml/min Extraction factor decreasing from 61 to 10

C Exp 610 317 III average plasma flow 74 ml/min Extraction factor decreasing from 51 to 13

	Time from injection min	Plasma radioactivity cps/ml		Total activity output in million counts		Added activity million counts	Added BSP mg/min
		Portal	Hepatic	Calculated from portal blood value	Found in hepatic vein blood		
A	3	5 257	7 162	0 805	1 340	0 535	1 6
	6	5 602	8 470	0 860	1 585	0 725	6 3
	10	6 866	7 902	1 050	1 480	0 430	3 7
	13	7 195	7 970	1 100	1 490	0 390	3 4
	16	6 607	8 410	1 015	1 570	0 555	4 8
	24	7 031	8 495	1 075	1 590	0 515	4 5
B	1	30,583	16 382	0 849	1 163	0 314	2 71
	4	24 315	18 729	1 101	1 330	0 229	1 98
	7	22 051	21 172	1 292	1 503	0 211	1 87
	10	22 119	19 988	1 412	1 419	0	0
C	2	24 679	42 250	0 855	3 000	2 145	18 5
	5	24 922	45 142	1 370	3 205	1 835	15 8
	16	21 651	38 936	1 336	2 764	1 478	1 3

the activity values have been corrected and 116 000 cps/mg for the second preparation Table II) it is possible from the increase in specific activity in the blood during a challenge experiment to calculate what fraction of the bromsulfalein has been added from the liver These values are minimum values as the specific activity in the liver in point of fact sinks through dilution with inactive bromsulfalein during the first minutes of the challenge experiment before samples have been taken This of course applies in a still higher degree when a second challenge experiment has been carried out immediately after the first

It emerges clearly from the tables that the specific activity in the venous blood of the liver is almost throughout higher than in the peripheral blood at the same point of time and that the challenge experiments entail a constantly rising admixture of radioactive bromsulfalein in the blood which is as a rule greater in the venous blood of the liver than in the portal blood

In order to ascertain whether the return of bromsulfalein from the liver can also be calculated from the difference between afferent and efferent blood

challenge experiments have been carried out simultaneously with the determination of the hepatic minute volume If p ml plasma per minute passes through the liver with the activity a impulses per second per ml in afferent blood then a total of p a impulses per minute are supplied Of this there passes a fraction which is the quotient for the bromsulfalein concentration in the hepatic venous blood and portal blood BSP /BSP The product of this quotient and the total amount of impulses supplied is the calculated total outgoing activity If the hepatic venous plasma has the activity a_{ex} then the actual outgoing total activity is p a and if this is significantly greater the surplus must come from the liver Such calculations have been made for 6 experiments, of which 3 are to be found in Table III The penultimate column shows an appreciable surplus activity from the liver

In these experiments the liver had absorbed a total of about 340 and 900 mg radioactive BSP respectively with a specific activity of 116 000 cps/mg If the bromsulfalein given off by the liver has the same high specific activity throughout the whole challenge experiment then to the activity surplus in the hepatic venous blood correspond the bromsulfalein amounts given in the last column of Table III

Discussion

From Table I and II it emerges clearly that the liver can give off bromsulfalein direct to the blood This is evidenced both by the higher specific activity in the hepatic venous blood and the stronger admixeture of radioactive bromsulfalein that can be calculated from the increase of specific activity with time as compared with the corresponding values for peripheral blood Since through continuous dilution with inactive BSP the specific activity of the bromsulfalein in the liver must decrease with time the calculated additions are rather too low than too high Yet the addition in the venous blood from the liver is not so inconsiderable as 10—15 min after the challenging bromsulfalein injection it amounts to between 4 and 60 per cent on an average 18 per cent of the total amount of bromsulfalein coming out on the venous side

Owing to individual differences in the experiments and the elements of uncertainty in the principles for the calculation one must take these figures only as a very approximative measure of the return of bromsulfalein from the liver to the blood In table III the reflux of bromsulfalein is calculated according to other principles which however also give minimum values Here too the calculated amount given off is considerable and between 1 6 and 32 per cent of the bromsulfalein absorbed by the liver If one calculates the cumulative minimum reflux of BSP to the blood during challenge experiments of between 16 and 25 minutes duration one obtains between 7 3 and 222 mg BSP If one calculates the decrease in specific activity in the

Table III Excess output of radioactivity in hepatic blood calculated from mean liver plasma flow and extraction factor Excess output in mg sulfobromophtalein/min based on specific activity of 116 000 cps/mg in the liver

A Exp 601,220 II liver plasma flow 187 ml/min extr factor 18 *

B Exp 610 317 II plasma flow 71 ml/min Extraction factor decreasing from 61 to 10

C Exp 610 317 III average plasma flow 74 ml/min Extraction factor decreasing from 51 to 13

	Time from injection min	Plasma radioactivity cps/ml		Total activity output in million counts		Added activity million counts	Added BSP mg/min.
		Portal	Hepatic	Calculated from portal blood value	Found in hepatic vein blood		
A	3	5,257	7 162	0 805	1 340	0 535	4 6
	6	5 602	8 470	0 860	1 585	0 725	6 3
	10	6 866	7 902	1 050	1 480	0 430	3 7
	13	7 195	7 970	1 100	1 490	0 390	3 4
	16	6 607	8 410	1 015	1 570	0 555	4 8
	24	7 031	8 495	1 075	1 590	0 515	4 5
B	1	30 583	16,382	0 849	1 163	0 314	2 71
	4	24 315	18 729	1 101	1 330	0 229	1 98
	7	22 051	21 172	1.292	1 503	0 211	1 87
	10	22 119	19 958	1 412	1 419	0	0
C	2	24 679	42,250	0 855	3 000	2 145	18 5
	5	24 922	45 142	1 370	3 205	1,835	15 8
	16	21 651	38,930	1 330	2 764	1 428	12 3

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In order to ascertain whether the return of bromsulfalein from the liver can also be calculated from the difference between afferent and efferent blood

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Department of Physiology Karolinska Institutet Stockholm and the Research Laboratories of AB Astra Sodertalje Sweden

Tenseness Variations in the Cat Kidney in Situ

By

A Åström and U Samelius

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Abstract

Åström A and U Samelius *Tenseness variations in the cat kidney in situ* Acta physiol scand 1962 55 35—44 — A previously described tonometer for continuous recording of kidney tenseness has been used to study intrarenal pressure changes under various experimental conditions The results have shown that tenseness (intrarenal pressure) is determined by the renal arterial and venous pressures the vascular tone and the colloid osmotic pressure of the blood Since intrarenal pressure increases with renal arterial pressure it is concluded that intrarenal pressure may tend to counteract the increase in blood flow which in other vascular beds is produced by a rise in arterial pressure However the question whether the intrarenal pressure plays a major role in the mechanism of true autoregulation of kidney blood flow seems to require further studies

The question whether variations in intrarenal pressure play a role in normal kidney function is still a matter of dispute Winton (1959) concluded that intrarenal pressure seems to play no important part in kidney blood circulation except possibly as an obstruction independent of arterial pressure in greatly swollen kidneys Swann and Prine (1951) on the other hand had proposed that intrarenal pressure which they found to increase with renal arterial pressure plays a major role in the control of kidney blood flow Hinshaw Day and Carlson (1959) and Scher (1959) have reported that the intrarenal pressure increases with renal arterial pressure and concluded that this may even explain the mechanism of autoregulation of kidney blood flow

In a recent communication (Åström Samelius and Strandell 1962) a tonometer for continuous recording of tenseness variations in the kidney *in situ* was described On the basis of the results obtained in anaesthetized cats it was concluded that the tenseness measured by this technique was a good estimate of intrarenal pressure

In the present investigation the factors determining kidney tenseness have been further studied and the results have been compared with intrarenal pressure measurements by previous authors Special attention has been given to the possibility that intrarenal pressure as measured by the tonometer, may play a role for the control of kidney blood flow

Methods

The tonometer used to measure the tenseness of the kidney is based on the same principle as the sclerotonometer and has been described in detail before (ÅSTRÖM *et al* 1961) The movements of the centrally located probe are recorded by means of an inductance pick up The whole tonometer is suspended by a pivoted arm balanced by springs so that no weight except that of the probe rests on the kidney The tonometer has three suction feet, 10 mm apart which hold the tonometer in place so that the movements of the probe are related to the surface of the kidney At the beginning of an experiment the sensitivity of the recording device including the amplifier is adjusted to a suitable range by occluding the arterial flow for 1—2 min (base line) and by elevating venous pressure to 20 and 30 mm Hg with an unhampered arterial blood supply

The experiments were done on a total of 32 cats anaesthetized with pentobarbital sodium (30—35 mg/kg) The left kidney was approached by a mid line incision and the tonometer applied after removing the peritoneum on the anterior surface of the kidney at the site of the suction feet Care was taken to keep the kidney moist and warm Intact circulation as well as constant volume perfusion was used

In the "intact circulation experiments the renal vein was cannulated and connected to the right femoral vein via a photoelectric drop counter or a Shipley Wilson rotameter Renal venous pressure was measured from a T tube close to the kidney and could be elevated by partial occlusion of the tubing distally to the flow measuring device

In the perfusion experiments the pump (Sigmamotor Inc) was inserted in a loop of polyvinyl tubing (Tygon) connecting the left renal artery to the right femoral artery Pressure fluctuations in the pump circuit were damped with a Windkessel Renal arterial pressure was recorded distally to the pump and venous pressure via a catheter inserted in the spermatic (ovarian) vein Venous pressure was elevated by partial occlusion of a screw clamp around the renal vein

When urine flow was recorded the renal pelvis was drained by a catheter introduced through the ureter The catheter outlet was fixed 10 cm below the zero plane for all pressure recordings (mid cardiac level) Urine drops were counted by a contact device and recorded cumulatively as a staircase curve obtained from a step relay set at zero at intervals of 0 5 or 1 0 min Systemic arterial pressure was obtained from a femoral artery Strain gauge transducers were used for all pressures (except tenseness) and all events recorded by a Grass Model 5 Polygraph Infusions were given and heparine injected into an external jugular vein

Results

The relationship between renal arterial pressure and tenseness was particularly studied in a series of experiments (12 cats) in which a pump was used to set the blood flow in the kidney at different levels Pressure flow relationships were studied by adjusting the flow rate and measuring the pressure distally to the pump

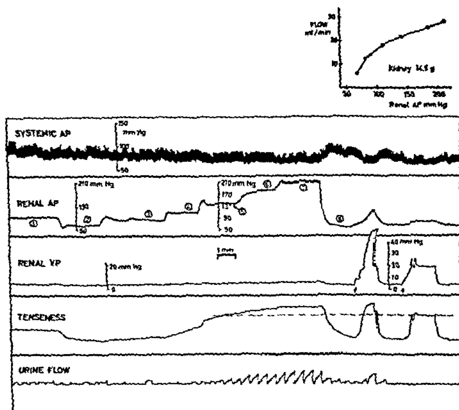


Fig 1 Cat 3 1 kg Systemic arterial pressure (AP) renal AP (perfusion pressure) renal venous pressure (VP) tenseness and urine flow recorded at different settings of the perfusion pump Tenseness increases with renal arterial (pump) pressure as does urine flow The pump flows were ① = 12 5 ml ② = 7 0 ml ③ = 12 5 ml ④ = 18 5 ml ⑤ = 2 0 ml ⑥ = 26 0 ml ⑦ = 28 0 ml and ⑧ = 7 0 ml/min.

At the end of the record venous pressure is elevated for calibration of the tonometer The diagram at the top shows the pressure flow relationships on the basis of values obtained 1 6 *min* after each new flow setting Weight of kidney 14 5 g

A typical experiment in this series is illustrated in Fig 1 The pressure flow curve at the top is based on pressure readings taken from the record at the end of the 1 6 min period at each flow setting The diagram indicates that the kidney shows autoregulation of blood flow i e at a pressure of about 100 mm Hg the slope is changed so that pressure rises sharply with only small increases in flow

From the record (Fig 1) it is evident that tenseness increases with blood flow and the concomitant elevation of the renal arterial perfusion pressure At the end of the record at a perfusion pressure of 70 mm Hg the venous pressure was elevated in order to check the sensitivity of the tonometer The

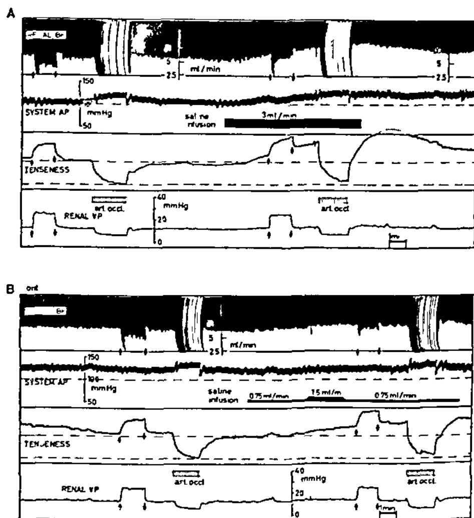


Fig 2 Cat 2 4 kg Intact arterial blood supply Renal blood flow (PF) recorded on the venous side as intervals between the drops Venous pressure (VP) is elevated to 25 mm Hg (between arrows) and the renal artery occluded before and during i v infusion at different rates of 0 9 NaCl solution For further explanation see text

increase in tenseness produced by this elevation of venous pressure to 20 mm Hg was the same as that previously observed at a perfusion pressure of 140 mm Hg and free venous drainage (venous pressure 4 mm Hg) The magnitude of a venous pressure which would produce the maximum tenseness observed at the highest perfusion pressure was technically difficult to determine Elevation of venous pressure to 30 or 40 mm Hg would frequently cause the suction feet of the tonometer to become disengaged From the practically total occlusion of the venous return for a short time prior to elevation of venous pressure to

20 mm Hg at the end of the record it is evident however that the tenseness at the perfusion pressure of 210 mm Hg and free venous outflow is of the same order of magnitude as that seen when the venous pressure is about 40 mm Hg at a perfusion pressure of about 70 mm Hg

An increase in tenseness with renal arterial pressure was regularly observed in the 12 cases in which renal arterial pressure was varied by the perfusion pump Thus tenseness definitely varied with renal arterial pressure and blood flow It was observed however that at the same renal arterial pressure tenseness was quite variable as expressed in terms of equivalent venous pressures In preparations with high resistance to flow (low flow value at maximum perfusion pressure) the increase in tenseness with increasing renal arterial pressure tended to be lower than in preparations with low resistance to flow

In a second series of experiments changes in tenseness of the kidney were studied during i v infusion of saline solution at different rates In a first group of experiments in this series intact arterial blood supply was used A typical result is illustrated in Fig 2 where A and B were recorded in direct succession Blood flow was recorded as intervals between blood drops (venous outflow) Repeated tests were performed by elevating venous pressure to 25 mm Hg and by complete occlusion of the renal arterial supply Tenseness increases with venous pressure elevation and falls when the arterial blood supply is occluded Before the second test in A i v saline infusion is started at a rate of 3 ml/min and as a result there is an increase in systemic arterial pressure kidney blood flow and tenseness When during the infusion the same venous pressure elevation (25 mm Hg) is performed tenseness increases to a higher level than before the infusion While here the venous pressure is maintained at 25 mm Hg tenseness continues to increase due to the i v infusion at the same rate as before the partial obstruction of the venous return Following complete occlusion of the renal artery the tenseness falls but after 1 6 min it is still somewhat higher than during the occlusion test prior to the saline infusion

In Fig 2 B similar tests are repeated when the different parameters have returned almost to control values and the response of tenseness is identical with that in Fig 2 A before the saline infusion The second pair of tests are performed during saline infusion at half the rate of that in Fig 2 A The results are qualitatively the same as in A *i e* the increase in tenseness produced by venous pressure elevation is added to that resulting from the saline infusion Following arterial occlusion the base line for tenseness is again somewhat elevated at the end of the 1 6 min occlusion period

Results similar to those in Fig 2 were consistently obtained in all 6 experiments with normal arterial blood supply of the kidney in which the infusion of saline or Ringer solution was performed at a rate sufficient to increase kidney tenseness Thus when the infusion increased the tenseness of the kidney venous pressure elevation caused an additional rapid increase of tenseness to

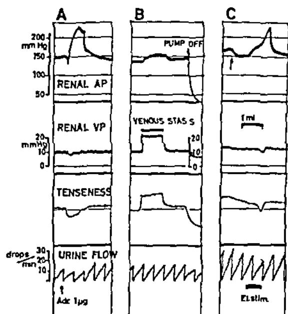


Fig 3 Cat 3 6 kg Kidney pump perfused with blood at constant rate

A Effect of close arterial injection of adrenaline (1 µg in 0 1 ml and flushed with 0 5 ml Ringer solution)

B Sensitivity check

C Electrical stimulation of renal nerves (4V and 5/s) At the arrow the pump was adjusted from 15 to 12 ml/min

Increasing vascular tone by both procedures in A and C increases renal AP and lowers tenseness

a level which always exceeded that produced by the same venous pressure elevation alone without simultaneous hemodilution Also the base line for tenseness during hemodilution, as observed after complete occlusion of the renal artery for 1 5—2 0 min was regularly elevated above the control level

Intravenous infusion of saline or glucose solutions in preparations with the kidney arterial blood supply maintained at a constant rate by the perfusion pump usually caused at least initially a decrease in the perfusion pressure as an indication of decreased peripheral resistance due to hemodilution During the same time tenseness usually remained fairly unchanged If in these experiments the perfusion rate was increased so that the same perfusion pressure as before the infusion was reestablished the tenseness of the kidney regularly increased Tests with elevated venous pressure under conditions of elevated tenseness in these experiments gave qualitatively the same results as in preparations with intact arterial blood supply *i e* the tenseness increase produced by a given venous pressure elevation was added to that produced by the hemodilution

In a third series of experiments the influence of acute changes in vascular tone on the tenseness of the kidney was studied in 10 preparations with constant rate perfusion as before Injections of adrenaline were made in the tubing proximally to the pump Electrical stimulation of the renal nerves was performed at a frequency of 5—8 cps obtained from a Grass Model S4 stimulator A typical experiment in this series is illustrated in Fig 3 The tracings top to bottom show renal arterial pressure renal venous pressure, renal tenseness

and urine flow In A, 1 μg adrenaline is injected intra arterially and causes an increase in vascular tone (increased arterial pressure at constant flow rate) and a decrease in tenseness Urine flow first decreases and then increases above control In B the sensitivity of the tonometer is checked in the usual manner In C finally the renal nerves are stimulated for about 45 sec Shortly prior to the stimulation the perfusion rate was lowered from 15 to 12 ml per min and as a result tenseness slowly decreased Electrical stimulation is followed by an increase in resistance and perfusion pressure rises sharply while tenseness and rate of urine flow decrease The effect is thus qualitatively the same as that of adrenaline injection in A

The results in this series of experiments illustrated that the renal arterial pressure *per se* did not determine kidney tenseness Results similar to those illustrated in Fig 3 were regularly observed in all the 10 preparations in which the influence of acute changes in vascular tone was studied

In the first series of experiments described above it was found that when blood flow varied urine flow increased with renal arterial pressure (perfusion pressure) as did kidney tenseness According to the results illustrated in Fig 3 however the urine flow recording did not run parallel with that of the renal arterial pressure but rather with the level of tenseness It may also be added that a secondary increase in urine flow after an initial decrease following adrenaline injection as in Fig 3 A was an almost regular finding

Discussion

In the pump perfused kidneys it has been shown that tenseness increases with perfusion pressure The level of tenseness at high perfusion pressures (about 200 mm Hg) is usually comparable to that seen at low perfusion pressures when the venous pressure is elevated to about 30—40 mm Hg It has previously been shown that in the kidney with intact circulation a partial occlusion of the renal artery is regularly followed by a fall in tenseness Thus tenseness is indeed dependent upon the renal arterial pressure This would as a matter of fact be expected if not only interstitial pressure but also the pressure inside the distensible vessels (veins and venules) and tubules determine tenseness and intrarenal pressure as discussed in the previous study (ÅSTRÖM *et al* 1962) The relationship between renal arterial pressure and tenseness is however not constant The degree of increase in tenseness with a given elevation of renal arterial pressure varied considerably from one preparation to another in the present investigation and also quite often in the same preparation at different times during an experiment This variability of the results would seem explicable by the finding that tenseness is determined not only by arterial pressure but also by the vascular tone (Fig 3) and the colloid osmotic pressure of the blood (Fig 2) In cases of high vascular tone a given renal arterial pressure would be expected to be associated with a relatively

low tenseness (intrarenal pressure) With low vascular tone in the kidney the same renal arterial pressure would be expected to be associated with a higher degree of tenseness

The conclusion that there is no direct relationship between renal arterial pressure and intrarenal pressure as reflected by the tenseness measurements is at variance with the conclusion by SWANN *et al* (1952) Our results are however in good keeping with those by MILES and DE WARDENER (1954) who concluded that intrarenal pressure, as they measured it by the needle puncture technique was dependent upon vascular tone as well as renal arterial and venous pressures

According to SWANN *et al* (1950) and GOTTSCHALK (1952) intrarenal pressure rises with venous pressure and above a value of about 20 mm Hg intra renal pressure (by the needle puncture technique) is found to be equal to venous pressure or to exceed it by less than 2 mm Hg Such a constant relationship between the two pressures does not apply before and during i v infusion of glucose or saline solutions (*Fig 2*) at least not if intrarenal pressure is studied in terms of tenseness

In a previous communication (ÅSTRÖM *et al* 1962) the intrarenal pressure was considered to represent a sum of interstitial pressure and the pressure, in excess of interstitial pressure prevailing in the distensible veins venules and tubules For flow to be possible through the kidney the pressure inside these structures must exceed the value of interstitial pressure (usually about 10—15 mm Hg) The pressure developing in these easily collapsible structures would be dependent upon renal arterial and venous pressures and also the vasomotor tone in the arterioles and small arteries (including the glomerular vessels with their afferent and efferent arteries) If this interpretation be correct it follows that there cannot be a constant relationship between one of the factors, *e g* renal arterial pressure and intrarenal pressure According to this concept the i v infusion of saline solution as in Fig 2 would cause an elevation of kidney tenseness by augmenting blood flow which would increase the pressure distension of the veins and venules An increased blood flow would be expected also at practically unchanged systemic (and renal) arterial pressure, as in Fig 2 b because of the decreased peripheral resistance during hemodilution A distension of the tubules due to an increased filtration rate and an elevation of true interstitial pressure as a result of increased net filtration into the tissue spaces as well as a distension of the lymph vessels would also contribute to an elevation of tenseness When in such a kidney the venous outflow is partially occluded the pressure in the intrarenal veins and venules will be further increased and thus a rapid increase in tenseness is added to that slowly produced by these other factors

A reproducible base line for the tenseness recording could usually be produced by occluding the arterial blood flow for 1 5—2 0 min During i v infusions of glucose or saline solution however arterial occlusion for the same

period of time did not lower tenseness to the control level (Fig 2) This would seem to indicate that during the infusion an increase in true interstitial pressure had occurred In a previous study of pressure time curves the minimum pressure for flow in the renal artery (yield pressure) was found to be determined by the intrarenal pressure (ÅSTRÖM 1960) Intrarenal pressure true interstitial pressure and intrarenal venous pressure would be expected to be equal at zero blood flow (ÅSTRÖM *et al* 1962) It would therefore seem that the base line for the tenseness recording as observed after 1 5—2 0 min occlusion of the renal artery could be given an absolute value if at the same time the intrarenal pressure was measured as yield pressure or intrarenal venous pressure

The blood flow governed by the arterial pressure and the vascular tone in the kidney would under ordinary conditions seem to control the intrarenal pressure by determining the pressure distension of the veins and venules (tubules) At high blood flows the further distension of these structures would be counteracted by the intrarenal pressure developing in the kidney with its limited distensibility An increased intrarenal pressure would however also tend to decrease the net distending pressure in the arterioles and small arteries This would tend to decrease the transsectional area of these vessels the resistance to blood flow would rise and as a result the pressure in the veins and venules would fall and the intrarenal pressure decrease In other words the prerequisites for a negative feed back mechanism would seem to be present in the kidney This conclusion would support the concept by HINSHAW *et al* (1959) SCHER (1959) and ÅSTRÖM and SAMELIUS (1960) The proposed mechanism would seem to be capable of maintaining blood flow relatively constant when fluctuations occur in the arterial pressure or the vascular tone in the kidney Further studies would seem to be required however before it will be possible to establish whether this feed back mechanism can fully explain the autoregulation of kidney blood flow *i e* the reason why at an arterial pressure of about 90—100 mm Hg the slope of the pressure flow curve of the kidney is changed so that with a further rise in arterial pressure flow increases only very little

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From the Department of Pharmacology University of Gothenburg
Gothenburg Sweden

Studies on the Effects of Drugs upon the Lactic Acid Metabolism and Contraction of Vascular Smooth Muscle

By

LENNART LUNDHOLM and ELLA MOHME LUNDHOLM

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Abstract

LUNDHOLM L. and E. MOHME LUNDHOLM *Studies on the effects of drugs upon the lactic acid metabolism and contraction of vascular smooth muscle* Acta physiol scand 1962 *55* 45—63 — The effects of contracting drugs — adrenaline noradrenaline histamine Ba^{++} ions — and electrical stimulation upon lactic acid production and muscle contraction were investigated under anaerobic conditions in experiments on bovine mesenteric arteries When the muscle contracted the lactic acid production was stimulated and the increase in production showed both a quantitative and a temporal correlation to the contraction After cessation of contraction the lactic acid production remained the same at different tone levels Substrate depletion attenuated and glucose augmented the contracting and lactic acid stimulating effects of drugs Glycolysis inhibiting substances and other metabolic inhibitors blocked the contractility under both anaerobic and aerobic conditions Calculation of the energy metabolism associated with contraction of mesenteric arteries indicated that the muscle's preformed high energy phosphate compounds sufficed only for a fraction of a total contraction. This behavior probably accounts for the apparently far greater dependence of vascular than of striated muscle upon a continuous energy production for the contractile processes The cause of the greater energy requirements for contraction of smooth than for contraction of striated muscle is discussed — Adrenaline stimulated glycolysis in vascular muscle even under aerobic conditions and in the presence of glucose Thus oxygen consumption alone cannot serve as a reliable indicator of the energy metabolism of smooth muscle

In an earlier paper (LUNDHOLM and MOHME LUNDHOLM 1960 a) we reported experiments on smooth muscle in which we studied the relation between tone (degree of contraction at a constant load) and energy production A distinct correlation was demonstrable between the energy production and the capacity of the muscle to maintain its tone at a certain level It was found however that while contracting agents such as histamine and barium led to stimulation of the metabolism during the initial contraction phase they subsequently enabled the muscle to reach a higher tone level at an unchanged energy production than was the case with untreated specimens

In the present investigation we studied in greater detail the relationship between the contractile process (increase of tone) and the energy production in mesenteric arteries The energy production was determined from the lactic acid production under anaerobic conditions This method has several advantages first it was possible to vary the energy producing capacity of the muscle by altering its carbohydrate supply secondly the synthesis of high energy phosphate compounds via anaerobic glycolysis probably bears a closer stoichiometric relationship to the lactic acid production than does the oxygen consumption since appreciable variations in the phosphorylation/oxidation ratio may occur Lastly a certain degree of glycolysis takes place in smooth muscle even under aerobic conditions (SUDHOF 1950 KIRK EFFERSOE and CHANG 1954 LUNDHOLM and MOHME LUNDHOLM 1960 a) and hence oxygen consumption alone is not a reliable criterion of energy production One shortcoming of determination of the energy metabolism from the lactic acid metabolism was that relatively long periods were required for the determination of the effects of drugs on the lactic acid production since these had to be calculated from differences in the lactic acid stimulating actions The effects of drugs to be reliably demonstrable must quantitatively exceed the biologic variation of the lactic acid stimulating actions hence the importance of the time factor Over short periods moreover the lactic acid production could not be expected to represent a dependable criterion of the energy metabolism since it had been found that the muscular content of preformed high energy phosphate compounds changed (LUNDHOLM and MOHME LUNDHOLM 1960 a) In view of these considerations we in no case determined the lactic acid production over periods shorter than 15 minutes

On the other hand mesenteric artery preparations showed very slow contraction — under certain experimental conditions maximal tone was not reached until at least 60 min had elapsed It is by no means certain therefore that determination of the lactic acid production over a shorter period would have shed greater light on our problem

The use of thermoelectric methods in determination of the effects of drugs on the energy metabolism of smooth muscle is unquestionably desirable but it appears that the technical difficulties involved have not yet been overcome (ABBOTT and LOWY 1958)

Method

The experiments were performed on bovine mesenteric arteries. A segment of artery approximately 15—20 cm long and with a uniform diameter of 3.5—4.0 mm was dissected free about 20 min after slaughter and transferred to iced Tyrode solution aerated with 93.5 % O_2 and 6.5 % CO_2. From each segment 10 to 16 specimens were prepared by first cutting the vessel longitudinally, then fashioning rectangular pieces 15 mm wide, 10—12 mm long and 0.8—1.00 mm thick with a weight of approximately 0.15 g. In the first few runs of experiments the preparations were attached simply by a hook at the upper and lower ends since the isotonic changes in length were being recorded, but we soon adopted the method of mounting the preparations in small plastic frames by means of three platinum hooks at each end, the load thus being equally distributed over the full width of the vessel. When the preparation had thus been mounted, care being exercised to avoid traction, it was transferred to an organ bath containing 20 ml Tyrode solution at 38° C previously saturated with N_2 or 93.5 % O_2 and 6.5 % CO_2. The changes of length were recorded by an isotonic pen with a load of 10.0 g and with a ratio of 1 : 10.

The Tyrode solution had the following composition (w/v): 0.8 per cent NaCl, 0.02 per cent KCl, 0.02 per cent $CaCl_2$, 0.02 per cent $MgCl_2 \cdot 2\,H_2O$, 0.1 per cent $NaHCO_3$, 0.005 per cent $NaH_2PO_4 \cdot 2\,H_2O$. The glucose concentration was varied as indicated in the following text. When this solution was aerated with 93.5 % O_2 and 6.5 % CO_2 it assumed a pH of 7.1, when aerated with N_2 a pH of 8.0. We elected to use the latter alkaline solution having observed that the artery preparations manifested an appreciably higher spontaneous tone in this medium than in the other solution. The lactic acid production of muscle under anaerobic conditions is known, moreover, to be greater in an alkaline than in an acid environment (KERLY and RONZONI 1933, HILL 1955). — It was desirable in these experiments to have as high anaerobic metabolism as possible.

The lactic acid production was determined as the difference between the total lactic acid content of a specimen and its Tyrode solution on conclusion of an experiment and that of an identically treated preparation assayed prior to the experiment. Duplicate tests were invariably performed. In this way we determined (1) the basal lactic acid production, (2) the effects of contracting drugs as well as in certain instances the effects of glycolysis inhibiting agents on that production and (3) the influence of those agents on the stimulation by adrenaline of the lactic acid production.

The effect of a drug on the lactic acid production was then recorded as the difference between the value for a treated preparation and that for an untreated control preparation from the same artery. The resulting data formed the basis of the statistical analysis for which the t test was employed (n = number of experiments, P = level of significance).

The lactic acid contents of preparation and of Tyrode solution were generally assayed separately by the method of FRIEDEMANN and GRAESER (1933) with the modifications reported by MOHME LUNDHOLM (1953).

Tonus alterations were recorded as millimeters of change relative to the initial length of the preparation immediately after mounting in the organ bath. Those differences in tone that were treated statistically had been determined, as a rule, at the conclusion of the experiment when the discrepancies were greatest. The relevant values included both the direct contraction produced by the drug and that inhibition of the spontaneous decrease in tone which, in general, was also present. Experiments determining contractile effect alone are explicitly designated in the text. Those tonus differences that were treated statistically are referable to different preparations from the same artery. Such an approach was necessitated by the fact that control preparations from

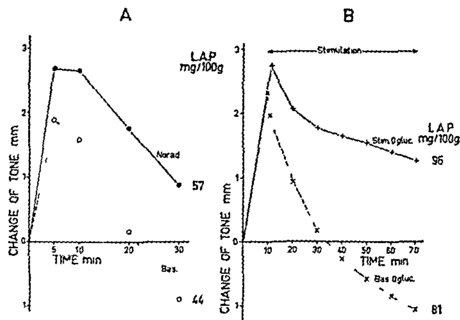


Fig 1 Effect of l noradrenaline 2 10⁻⁴ at 1 and 15 (A) and electrical stimulation 15 V 50 cps for 16 min (B) on tone and lactic acid production (L A P) of bovine mesenteric artery Tyrode solution Without glucose in N

different arteries exhibit spontaneous alterations of tone which in turn show e g seasonal variability (LUNDHOLM and MOHME LUNDHOLM 1960 a) — The observed tonus changes thus reflected variation in the muscle activity and not — as in experiments on striated muscle — a transition from rest to activity

Isolated rabbit ear was perfused with Tyrode solution containing 0 1 per cent glucose and aerated with 93 5 % O_2 and 6 5 % CO_2 These experiments were carried out at 22 C and the flow was recorded by drop recorder

Adrenaline and other drugs were added at 15 minute intervals unless otherwise stated in the text the purpose being to replace any substance that may have been inactivated and to ensure a maximally active concentration in the organ bath

Electrical stimulation of preparations was produced via platinum hooks in Tyrode solution by alternating current 50 cps and 15 V — the lowest voltage that gave a maximal effect The current strength was approximately 0 1 Amp but the greater part of the current probably passed through the Tyrode solution

Results

Lactic Acid Production and Increase of Tone after Drugs and Electrical Stimulation in the Absence of Glucose

Anaerobic Conditions In the first series of experiments we studied the effects of various stimulating agents on tone and lactic acid production under anaerobic conditions An initial dose of the relevant drug was added one minute after mounting of the preparation and a second dose after 15 min The dura

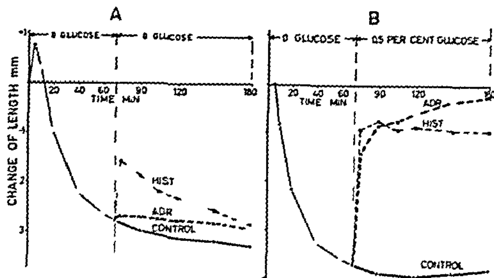


Fig 2. Effects of adrenaline and histamine on tone and lactic ... duction of substrate depleted mesenteric artery After 60 min in glucose free Tyrod... the solution was exchanged for glucose free Tyrode (A) or Tyrode with 0 ... se (B) and ad renaline (1 10⁻⁶) or histamine (5 10⁻⁶) was added at 15-min ...

Table II Tests on substrate-depleted mesenteric artery Afte ... Tyrode solution adrenaline (1 10⁻⁶) or histamine (5 10⁻⁶) was added at ... second series glucose (0 5 per cent) also was added at 60 min. The ex ... e performed in May and those with glucose in August which ma ... n the two series with regard to control lactic acid production and ... olm and Mohme Lundholm, 1960 a.

Test	Lactic acid production mg/100 g		
	0–60 min no glucose	60–180 min. control	Increase 60–180 Adrenal
With no glucose (n = 7)	61.8 ± 8 1	26 7 ± 7 1	3 9 ± 3
With glucose at 60 (n = 6)	34 1 ± 13 9	170 9 ± 27.2	70 1 ± 11 1 P < 0 01

Aerobic Conditions In one run of experiments renaline on tone and lactic acid production conditions were compared. Both the spontaneo contractile conspicuou aline ditions.

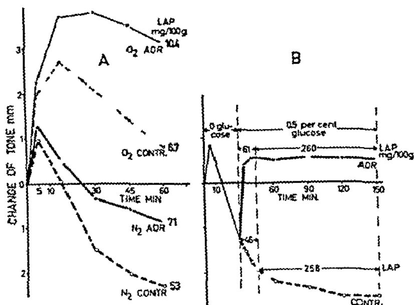


Fig 3 A Effect of adrenaline (1 10^{-6} at 15 minute intervals) on tone and lactic acid production under anaerobic (N_2) and aerobic (93 5 per cent O + 6 6 per cent CO) conditions Tyrode solution without glucose

B Lactic acid production during contraction and period of increased tone After 30 min in glucose free Tyrode the solution was exchanged for Tyrode with 0 5 per cent glucose and adrenaline 1 10^{-6} was added at 15-minute intervals The lactic acid production as determined for the periods 30—45 min and 30—150 min, is indicated in the diagram

duction by 18 1 ± 5 2 mg/100 g/70 min ($n = 6$ $P < 0\ 01$) Under aerobic conditions the arteries produced a certain amount of lactic acid (6 7 ± 2 8 mg/100 g/70 min $P < 0\ 05$) and although this production rose somewhat after adrenaline (10 4 ± 4 0 $P < 0\ 05$) the increase was not statistically significant Compare however the experiments with glucose under aerobic conditions

Lactic Acid Production and Elevation of Tone after Adrenaline and Histamine in the Presence of Glucose

Inaerobic Conditions The augmented contractile effect of adrenaline under aerobic conditions suggested that the energy production of vascular muscle influenced the magnitude of the tone increase Experiments were therefore conducted in which we studied the influence of glucose on the contractile response and the lactic acid production following administration of adrenaline and histamine

In an initial run of experiments 0 5 per cent glucose was added immediately after mounting of the preparations and the effect of adrenaline was compared with that occurring in glucose free preparations from the same artery The contractile effect of adrenaline — added one minute after mounting — was

1 12 ± 0 45 mm ($n = 7$ $P < 0$ 05) greater in the experiments with glucose than in those without The tonus differences were, in this instance measured 10 min after the addition of adrenaline Even the effect of adrenaline on the lactic acid production appeared to be augmented in the experiments with glucose, where the rise amounted to 40 6 ± 14 4 mg/100 g/70 min, as against 23 0 ± 7 9 in the glucose free experiments

The effects of glucose on the contracting and lactic acid stimulating actions of adrenaline and histamine were more distinct when the muscle had first consumed a part of its own substrate reserves whereby the initial tone of the preparations was low When preparations were initially mounted in glucose free Tyrode solution and the latter was exchanged after 60 min, for solution containing 0 5 per cent glucose the effects of adrenaline and histamine were far more pronounced than in the absence of glucose (Fig 2 B Table II) Although glucose *per se* stimulated the lactic acid production of the control preparations to a much higher degree than did adrenaline or histamine its effect entailed merely a reduction or cessation of the tone decline and no demonstrable contraction When on the other hand aerobic conditions were applied the artery contracted (LUNDHOLM and MOHME LUNDHOLM 1960 a) Hence aerobic and anaerobic energy production seem to differ in their effects on tone

In another series of experiments we studied the relative time course of lactic acid production and elevation of tone following adrenaline administration (Fig 3 B) After immersion of the preparations for 30 min in glucose free Tyrode solution the latter was exchanged for solution containing 0 5 per cent glucose concurrently with addition of adrenaline to every second preparation The lactic acid production was determined both after 15 min, when the contracting effect of adrenaline had almost reached its maximum and after 120 min The difference in lactic acid production between the adrenaline and the control tests was 14 9 ± 5 6 mg/100 g ($n = 7$ $P < 0$ 05) after 15 min and 17 4 ± 25 0 mg/100 g after 120 min The stimulation of the lactic acid production thus coincided with the contraction during the first 15 min and was also reflected though not significantly in the values recorded after 120 min When the contraction had practically terminated the lactic acid production was 257 5 ± 27 2 mg/100 g/15—120 min in the control tests and 260 2 ± 29 2 or virtually equivalent in the adrenaline tests — This run of experiments with adrenaline substantiates previous findings with histamine and Ba^{++} ions (LUNDHOLM and MOHME LUNDHOLM 1960 a) namely that lactic acid production is only stimulated initially in connection with the increase of tone and that an elevated tonus level can subsequently be maintained without further augmentation of the energy production

During the first 15 min after addition of glucose in the control experiments the lactic acid production was on the average, 9 4 mg/100 g higher than during a subsequent 15 min period It may be assumed that this extra lactic

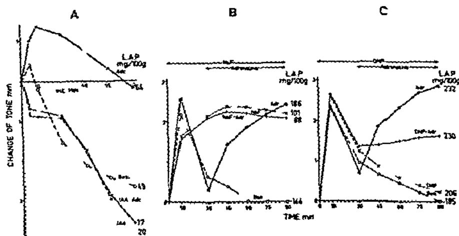


Fig 4 Effect of metabolic inhibitors upon the stimulating action of adrenaline on tone and lactic acid production

A Mono-iodoacetic acid (0 022 M) was added at 1 Adrenaline was added at 10 25 40 and 55 Test without glucose

B Sodium fluoride was added in a dose of 5 mg/20 ml at 1 10 20 and 30 total concentration 0 024 M Adrenaline (1 10⁻⁶) at 30 45 60 and 75

C Dinitrophenol (0 0054 M) was added at 1 adrenaline at 30 45 60 and 75

acid production was utilized in resynthesizing those high energy phosphate compounds which the muscle had consumed during the earlier period of substrate depletion The formation of 1 mol lactic acid from glucose is associated (KAPLAN 1951) with concomitant synthesis of 1 eq high energy phosphate bonds (= 1 eq ~ ph) By virtue of the extra lactic acid production therefore 1 05 μeq/g ~ ph would be formed On direct assay of the increase in high-energy phosphate compounds occurring in mesenteric arteries under experimental conditions identical with those in the above mentioned runs we found an increase of 1 1 μeq/g ~ ph (LUNDHOLM and MOHME-LUNDHOLM 1960 b)

Aerobic Conditions Glucose augmented the lactic acid stimulating effect of adrenaline even under aerobic conditions For one run of experiments in which Tyrode solution with 0 1 per cent glucose was used the lactic acid production of the control preparations was 18 3 ± 5 8 mg/100 g/90 min ($n = 12$ $P < 0\,01$) On addition of adrenaline the glycolysis increased to a level exceeding that for the control preparations by 15 4 ± 5 4 mg ($P < 0\,05$) The maximum elevation of tone in the adrenaline experiments was 1 2 ± 0 4 mm ($P < 0\,01$) higher than the spontaneous rise of tone in the controls It may be estimated — see below — that approximately 50 per cent of the energy required for this contraction could have been obtained from glycolytic reactions — a circumstance which demonstrates that determination of the oxygen consumption alone is not a reliable indicator of the energy metabolism of smooth muscle

Table III. Effects of metabolic inhibitors on the stimulating action of adrenaline $(1:10^{-6})$ at 15-

Drug concentration	Glucose per cent	Duration min.	n	Difference in lactic acid production mg/100 g	
				1	2
				Adrenaline—control	Drug—control
Cu++ (0.003 M)	0	70	7	23.0 ± 7.9 P < 0.95	8.2 ± 5.0
Cu++ (0.003 M)	0.5	70	7	40.6 ± 14.4 P < 0.05	4.4 ± 10.2
Iodoacetic acid (0.022 M)	0	70	5	15.3 ± 3.1 P < 0.05	−28.5 ± 9.8 P < 0.05
NaF (0.024 M)	0.5	90	5	43.1 ± 8.5 P < 0.01	−42.4 ± 14.1 P < 0.05
Dinitrophenol (0.0054 M)	0.5	90	5	49.6 ± 10.7	22.8 ± 25.0

Effects of Glycolysis Inhibiting Drugs on the Contracting and Lactic Acid Stimulating Actions of Adrenaline

Anaerobic Conditions. In experiments hitherto reported an elevation of tone has invariably been associated with stimulation of the carbohydrate metabolism but the question arises as to whether vascular muscle like striated muscle is not capable of numerous contractions by utilization of its preformed high energy phosphate compounds. With this question in mind we investigated the effects of certain glycolysis inhibiting substances which, in previous experiments (MOHME LUNDHOLM 1953, LUNDHOLM and MOHME LUNDHOLM 1957), had been observed to depress the augmentation by adrenaline of lactic acid production and glycogenolysis in smooth muscle.

In one run of experiments with glucose free Tyrode solution we added mono-iodoacetic acid as sodium salt adjusted to pH 7.4 in a concentration of 0.022 M immediately after the preparations had been mounted and 10 min after addition of adrenaline. Mono-iodoacetic acid reduced the lactic acid production to 45 per cent of that of the control preparations and totally suppressed both the initial elevation of tone and the contracting and lactic acid stimulating effects of adrenaline (Fig. 4 A. Table III).

Glycolysis was in mesenteric arteries appreciably more resistant to mono-iodoacetic acid than it was in striated muscle. In experiments on isolated rat diaphragm we found that treatment with mono-iodoacetic acid at a concentration of 0.00018 M for 20 min under anaerobic conditions depressed the glycolysis by over 90 per cent both in the presence and in the absence of glucose

minute intervals on tone lactic acid production of mesenteric artery

Difference in lactic acid production mg/100 g		Difference in tone (mm) on conclusion of experiment		
3 / DrugAdrenaline —drug	Difference 1—3	Adrenaline—control	Drug—control	DrugAdrenaline —drug
−9 5 ± 2 9 P < 0 05	32 5 ± 10 0 P < 0 05	2 24 ± 0 49 P < 0 01	0 1 ± 0 32	0 ?4 ± 0 24
−11 2 ± 7 4 P < 0 05	51 8 ± 20 7 P < 0 05	3 76 ± 0 63 P < 0 001	0 74 ± 0 49	0 16 ± 0 31
−4 0 ± 5 3	19 2 ± 7 9 P < 0 05	2 5 ± 0 45 P < 0 001	−1 2 ± 0 38 P < 0 05	0 25 ± 0 31
−16 7 ± 7 4	59 9 ± 15 5 P < 0 05	2 56 ± 0 61 P < 0 01	2 23 ± 0 42 P < 0 001	−0 13 ± 0 39
24 7 ± 15 8	24 9 ± 21 2	2 61 ± 0 22 P < 0 001	0 24 ± 0 35	1 34 ± 0 33 P < 0 01

Isolated rabbit ear seems however to be somewhat more sensitive than mesenteric artery to mono iodoacetic acid (see below)

b Sodium fluoride in a concentration of 0 024 M reduced the glycolysis to about 70 per cent of that of the control preparations in experiments with 0 5 per cent glucose Even the effect of adrenaline on lactic acid production was depressed Since sodium fluoride *per se* influenced the tone — it counteracted the tonus reduction in the control preparations — it was difficult to ascertain whether adrenaline s effect on the tone was also blocked Worthy of note is the very slow elevation of tone following addition of adrenaline in these experiments (Fig 4 B)

Cu^{++} ions in a concentration of 0 003 M added immediately after mounting of the preparations and 10 min before the first adrenaline dose inhibited the contracting as well as the lactic acid stimulating actions of adrenaline in Tyrode solution both with 0 5 per cent glucose and without glucose (Table III) Since however Cu ions did not inhibit the spontaneous glycolysis it is problematical whether they exerted a non specific glycolysis blocking effect or a more specific adrenolytic action The possibility that Cu^{++} ions inhibited adrenaline via increased oxidation (STARKENSTEIN 1941 CHAIX MORIN and JEZEQUEL 1950) seems inadmissible since the experiments were performed in the presence of N_2

Dinitrophenol is assumed to have an uncoupler effect in oxidative phosphorylation but at fairly high concentrations it also interferes with the carbohydrate metabolism (SIMON 1953) — At a concentration of 0 0054 M di-



Fig. 5. A. Mesenteric artery. Tyrode solution aerated with 93.5 per cent O_2 – 6.5 per cent CO_2. Sodium azide 0.0077 M was added 15 min before the first dose of adrenaline. (1) Adrenaline 1 : 10^{6}. (2) adrenaline 5 : 10^{6}. Upper curve, test without glucose. Lower curve 1 per cent glucose present.

B. Isolated rabbit ear perfused with Tyrode solution containing 0.1 per cent glucose aerated with 93.5 per cent O_2 – 6.5 per cent CO_2. (1) 0.1 µg adrenaline. (2) 20 mg dinitrophenol. (3) 0.1 µg adrenaline.

C. Isolated rabbit ear. "Sympathetic tone" had been produced by adding l-noradrenaline 1 : 10^{8} to the Tyrode solution. At the arrow 8 mg DL-glyceraldehyde.

nitrophenol conspicuously depressed the contracting effect of adrenaline without inhibiting the spontaneous glycolysis in arterial muscle (Fig. 4 C, Table III).

Aerobic Conditions. It was of interest to determine whether those substances which in various ways interfered with glycolysis even under aerobic conditions, depressed the contracting effect of adrenaline, histamine, acetylcholine, and Ba^{++} ions. They were, in fact, found to do so. In addition to these substances we used, in the same experiments, DL-glyceraldehyde (0.04 M) and sodium azide (0.0077 M). An interesting find was that the blocking action of sodium azide could be inhibited by addition of glucose. The higher the glucose concentration, the greater was the inhibition (Fig. 5 A).

One general objection which could be raised against the experiments with metabolic inhibitors is that total block was contingent upon a relatively long interval between administration of the inhibiting and the contracting substances, a circumstance probably attributable at least in part, to slow diffusion of the metabolic inhibitors into the prepar ... use of the latter's thickness. — It was to be ... the bulk o... e could, in the meantime, have consumed its ... compounds for its basal metabolism. As w... d production from glycogen and int... ons was approximately 0.2 ... The available ... are not likely to ex... With the s... the catabolism which ...

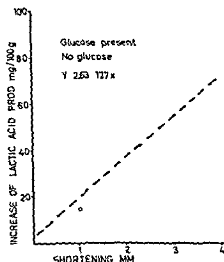


Fig 6 Correlation between the shortening in millimeters (x) and the increase of lactic acid production (y) attributable to different drugs and electrical stimulation test without glucose test with 0 5 per cent glucose

Isolated Rabbit Ear Experiments with metabolic inhibitors were therefore performed on a tissue which could be expected to show a more favourable diffusion behavior than mesenteric arteries namely isolated rabbit ear, which was perfused with oxygenated Tyrode solution containing 0 1 per cent glucose In this preparation the various metabolic inhibitors had the same depressive action on contracting substances as that observed in experiments on mesenteric arteries without sodium fluoride which potentiated the effect of adrenaline Dinitrophenol completely suppressed the action of adrenaline 3 5 min after administration (Fig 5 B) while DL-glyceraldehyde was able to counteract instantaneously the sympathetic tone induced by addition of noradrenaline to the perfusion solution (Fig 5 C) Mono iodoacetic acid acted less rapidly not until perfusion with Tyrode solution containing 0 0027 M mono iodo acetic acid had proceeded for 20 min was the effect of adrenaline totally suppressed while that of histamine was appreciably reduced

Correlation between Lactic Acid Production and Muscle Contraction

Since the period of lactic acid stimulation coincided with that of the tonus elevation the question arose as to whether these two effects were correlated quantitatively as well In Fig 6 that increase of the lactic acid production over the basal value which was produced by drugs or by electrical stimulation is correlated with the contraction induced by various tone promoting factors Disparities between control and treated preparations with regard to the relaxation rate are not allowed for in these values Irrespective of differences in experimental method and type of stimulation in the various runs of experiments a statistically significant correlation was demonstrable between con

raction in mm and lactic acid increase in mg/100 g From the correlation ine equation $Y = 2\ 63 - 17\ 74\ X$ ($r = 0\ 710$ $P < 0\ 001$) it was evident both hat the muscle produced, per mm of contraction, an average of 17 7 mg lactic acid per 100 g, and that the lactic acid increase tended to approach zero when he muscle failed to contract. This correlation includes however both those experiments in which lactic acid was produced solely from glycogen and inter nediate products and those in which glucose constituted the substrate. The ynthesis of high energy phosphate compounds is 1 5 times as high per mol of lactic acid formed from glycogen or intermediate products as for that produced from glucose (KAPLAN 1951) Hence the lactic acid production per mm of contraction should, logically, be lower for preparations in glucose free Tyrode solution than for those in Tyrode solution containing glucose. And his indeed, appeared to be the case In glucose free Tyrode solution the average quotient for lactic acid production in mg/100 g per mm contraction was 15 7 ± 4 0 ($n = 7$) but in Tyrode solution with glucose it was 1 46 times as high or 23 0 ± 3 3 ($n = 13$)

It was probable that the spontaneous contraction of the control prepara ion was also combined with a stimulation of the lactic acid production. We found that pretreatment in cold for 30 min with dibenamine at a concentra ion of 5 10^{-4} completely suppressed the spontaneous contraction, which in he control preparations averaged 1 17 ± 0 37 mm at the same time diben reduced the lactic acid production of the preparations by 26 0 ± 5 3 mg 100 g The experiments were performed in the presence of 0 5 per cent glucose The quotient of lactic acid production contraction was calculated at 22 2 which closely accords with the mean of 23 0 for experiments in the presence of glucose The possible mechanism of this effect of dibenamine is discussed in a subsequent paper

Lactic Acid Production and Work Performed

It may be asked to what degree the lactic acid production depended on the work performed by the muscle This question was studied by carrying out a series of experiments in which the preparations mounted in glucose free Tyrode solution were initially subjected to a load of only 2 g. After 5 min the load on every second preparation was raised to 20 g After a further 35 min the solution was exchanged for Tyrode solution containing 0 5 per cent glucose and at the same time histamine (5 10^{-5}) was added. The dose was repeated after 60 min During the first 35 min in glucose free Tyrode solution the lactic acid production was not verifiably higher in preparations with the lighter than in those with the heavier load. The latter preparations had, in the presence of glucose however a lactic acid production which exceeded by 57 4 ± 18 9 mg 100 g 120 min $n = 7$ $P < 0\ 05$) or 29 per cent, that of the preparations with a load of only 2 grams On addition of histamine to the lightly loaded preparations the latter performed, on contraction 0 54 gcm

work and their lactic acid production increased by 64 2 ± 22 2 mg/100 g ($P < 0$ 05) The corresponding figures for preparations with the heavier load were 8 1 gcm and 54 8 ± 21 3 mg/100 g ($P < 0$ 05) respectively Thus there was no demonstrable correlation between work performed and lactic acid production

Discussion

The close correlation between those tone increasing and lactic acid stimulating effects of drugs which emerged in the experiments on arterial muscle may be accounted for in various ways which will be further discussed in a subsequent report (LUNDHOLM and MOHME LUNDHOLM to be published) The *a priori* most plausible assumption however is the classical one according to which the stimulation of the metabolism results from that hydrolysis of high energy phosphate compounds which is thought to accompany the contractile process The lactic acid production should on this assumption be a criterion of the metabolic rate of these high-energy phosphate compounds In the following discussion this theory is accepted as a basis of approach to the correlation between contraction and lactic acid stimulation. As for the content of high energy phosphate compounds in vascular muscle the discussion will be confined to adenosine triphosphate (ATP) adenosine diphosphate (ADP) and creatine phosphate (Cr P) although these substances are probably neither the sole suppliers of energy (HILL 1955) nor those primarily utilized (DAVIES CAIN and DELLUVA 1959)

The first question that arises is why the relationship between muscle contraction and stimulation of the metabolism appears to be so much closer in smooth than in striated muscle Although the contracting effect of *e g* adrenaline or histamine on vascular smooth muscle cannot apparently be dissociated from the carbohydrate stimulation under the influence of various metabolic inhibitors the same does not apply to striated muscle Striated muscle of frog which has been so treated with mono-iodoacetic acid as to block the glycolysis may still in the presence of N_2 be capable of a hundred or so contractions (LUNDSGAARD 1930)

Energy Metabolism Associated with Contraction Estimation of the energy metabolism associated with contraction of vascular muscle in relation to the muscle s content of preformed high energy phosphate compounds is of value in this context

It may be estimated that with a contraction of 1 mm the lactic acid production from glycogen and intermediate products amounted to 1 74 μM per g muscle If 1 mol lactic acid is produced from glycogen or intermediate products the net synthesis will be 1 5 eq ~ph (KAPLAN 1951) The muscle accordingly synthesized 2 5 eq/g ~ph concomitantly with lactic acid production Mesenteric arteries immersed for 30 minutes in oxygenated Tyrode solution with 0 1 per cent glucose contained on the average ($n = 7$) 2 55 μeq/

g ~ ph distributed as follows 0 68 μM/g ATP = 1 36 μeq ~ ph 0 87 μM/g ADP, and 0 52 μM/g Cr P (LUNDHOLM and MOHME-LUNDHOLM 1960 a) Assuming that the high energy phosphate compounds which coincided with lactic acid production were consumed on contraction — as, indeed, has been demonstrated experimentally (LUNDHOLM and MOHME LUNDHOLM to be published) — *it may be estimated that the muscle s preformed high energy phosphate* compounds if fully utilized would have made possible a contraction of 1 mm

This, however is but a fraction of the total contractile power of vascular muscle for mesenteric arteries were able, from their basal initial length of approximately 10 mm to relax to about 15 mm on treatment with monoiodoacetic acid or after extreme substrate depletion (LUNDHOLM and MOHME LUNDHOLM 1960 a) or to contract to about 6 mm in oxygenated, glucose containing Tyrode solution and after addition of contracting drugs The total potential contractility of the arterial muscle was thus approximately 9 mm — experimentally observed, 7 mm — or in other words the muscle was able to contract to 40—50 per cent of its fully relaxed length

From these values it may be estimated that mesenteric arteries would need to consume for a maximum contraction of 9 mm about 23 μeq/g ~ ph, which is 9 times as much as the preformed amount

Comparison with striated muscle of frog is of interest in this connection On maximal tetanoid contraction, muscle may contract to 60 per cent of its [illegible] l length and it may be estimated from the heat production that approximately 0 25 μeq/g high energy phosphate compounds is concomitantly broken down (HILL 1950) DAVIES CAIN and DELLUVA (1959) demonstrated an increase of about 0 5 μM/g in the content of inorganic phosphate with concurrent hydrolysis of an as yet unidentified phosphoric acid ester The total content of identified high energy phosphate compounds in frog muscle, assayed as in the case of mesenteric arteries corresponded to 17 4 μeq/g ~ ph (2 8 μM/g ATP 0 7 μM/g ADP 11 1 μM/g Cr P) (FLECKENSTEIN *et al* 1954) Frog muscle may therefore be estimated to consume with a maximal contraction *approximately 3 per cent of its (known) preformed high energy phosphate* compounds

These computations suggested that the contractile process in vascular muscle required so much energy that only a fraction of that needed for a maximal contraction could be supplied by the muscle s preformed high energy phosphate compounds The muscle was thus dependent on a continuous energy production for its contractile activity This circumstance could account for the *apparently far more intimate relationship between* contraction and energy production in smooth than in striated muscle

Cause of the Energy Demand If the above assumption is correct the question which arises is why the contractile process requires so much more energy in smooth than in striated muscle According to the thermal studies of HILL and coworkers (for review *vide* WILLKIE 1954) those energy requiring reactions

which are associated with the contractile process in muscle may be attributable to external work activation heat shortening heat and recovery heat The lactic acid production is probably the metabolic equivalent of the sum of these energy consuming processes One may ask however whether the difference between vascular and striated muscle can be ascribed to a single component and if so which one — It was thus of interest to study the significance of the various components in the lactic acid production

External Work The arterial preparations were 15 mm wide, 10 mm long and 1 mm thick and weighed approximately 0 15 g They had a load of 10 g and at a contraction of 1 mm performed work of 1 gcm, the thermal equivalent of which was 2 3 10 $^{-5}$ gcal On the average the muscle produced 15 7 mg lactic acid per 100 g from glycogen and intermediate products or 10 5 μg/0 15 g The production of 1 g lactic acid from glycogen is associated with an energy production of 390 cal (DICKENS 1951) Of the increased energy production of 4 1 10 $^{-3}$ cal/0 15 g muscle only 0 56 per cent therefore could be attributed to external work These computations may it would seem account for the lack of any appreciable difference between the lactic acid production on contraction at a load of 2 g and that on contraction at a load of 20 g

Recovery heat is under anaerobic conditions only about 1/20 of the initial heat (activation and shortening heat combined) evolved during the contractile process itself (HILL 1940) It seems unlikely that the anaerobic chemical processes underlying the recovery heat in vascular muscle would diverge from corresponding processes in striated muscle to such a degree as to account for the major disparities in energy metabolism

Shortening Heat It is tempting to assume that the observed correlation between lactic acid production and contraction in mm is the metabolic equivalent of HILL s (1938) heat of shortening — We therefore calculated the magnitude of the constant *a* from the lactic acid production and obtained a value of 26 700 g wt/cm^2 muscle surface area HILL in experiments on frog muscle recorded a value of 400 wt/cm^2 If this high value for *a* were accepted it would serve to explain why the contraction required so much energy in vascular muscle Dissociation of the contracting from the lactic acid stimulating effects of drugs on vascular muscle has been demonstrated however under certain experimental conditions (LUNDHOLM and MOHME LUNDHOLM to be published) — a result which suggests that the lactic acid production cannot serve as a criterion of shortening heat

Activation Heat A more plausible explanation of the high energy metabolism associated with contraction of vascular muscle is that the increased lactic acid production results from that excitative process which in turn leads to muscle contraction The lactic acid production would on this assumption be a counterpart of activation heat Mesenteric arteries contract very slowly — at a maximum initial rate of about 2 mm/min when not loaded — and hence prolonged excitation is required for the muscle contraction to reach its maxi

mum Since frog muscle contracts much faster — at a rate of approximately 240 mm/min when not loaded (HILL 1938) — it is evident that here the duration of excitation may be considerably shorter and the total energy metabolism substantially lower than in vascular muscle

Calculation of that lactic acid production which may be referable to activation heat is impracticable on the basis of available data, since its magnitude is probably a function of the muscle length There is ample evidence that drugs which heighten the tension in smooth muscle under isometric conditions also stimulate the heat production (ABBOTT and LOWY 1958) and increase the oxygen consumption (BULBRING 1953) experiments which demonstrate the presence of activation heat in smooth muscle

On the other hand it was found in our experiments that when the muscle had contracted under the influence of drugs the lactic acid production did not diverge from the metabolism of the control preparations No metabolic equivalent of activation heat was then demonstrable

If the stimulating effects of drugs on the lactic acid production of smooth muscle result from the excitative process smooth muscle which contracts more rapidly than vascular muscle should require less energy for contraction under isometric conditions the lactic acid production after administration of drugs should, moreover be a function of the muscle length Further experiments are required to substantiate this hypothesis

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From the Division of Food Chemistry Royal Institute of Technology and the Departments of Physiology and Clinical Biochemistry Royal Veterinary School, Stockholm, Sweden

The Loss of Added Lysine During the Baking of a Soft Bread with an Exceptionally High Content of Reducing Sugar

By

L. E. ERICSON and S. LARSSON

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Abstract

ERICSON, L. E. and S. LARSSON. *The loss of added lysine during the baking of a soft bread with an exceptionally high content of reducing sugar.* Acta physiol. scand. 1962. 55. 64—73. — An estimation was made of the loss of supplementary L-lysine HCl that occurs during the baking of a soft bread containing a very high content of syrup. Rats and bacteria served as test systems. The nitrogen efficiency ratios of young albino rats given bread diets to which L-lysine HCl was added before or after baking indicated that approximately 30 % of the L-lysine HCl added before baking was inactivated or destroyed. Extraction of the supplementary L-lysine HCl with cold dilute acid or alkali followed by microbiological determination of the lysine gave values of the order of 25 % for the loss of lysine during baking.

It was found that the protein value of the type of bread studied was in itself poor, probably due to inactivation of the protein-bound lysine caused by reactions with the reducing sugar.

Liver fat content and three different liver transaminase activities were also measured. No pathological changes were indicated.

In a previous paper we reported the loss of added lysine and threonine during the baking of wheat bread (ERICSON, LARSSON and LID 1961a). It was found that the loss of lysine was rather small — 10—15 % as estimated by animal experiments and 5—10 % as estimated by microbiological assays — whereas the loss of threonine appeared to be considerably greater. In a

subsequent study of the efficiencies of free L-lysine HCl roller-dried skim milk, fish protein and soya bean protein as supplements to wheat bread we noticed that the supplementary value of the dried milk was not in proportion to its microbiologically determined content of lysine which under the conditions of the experiments was true for the other supplements (ERICSON, LARSSON and LID 1961b). This indicated that part of the lysine provided by the milk supplement had become biologically unavailable during the baking, probably due to reactions with the milk sugar. Such an interpretation would be in accordance with the numerous reports dealing with losses of protein bound lysine caused by condensations with sugars (cf HARRIS and LOESECKE 1960).

It is common practice in Sweden to add considerable quantities of syrup to certain types of soft bread. In one such bread which accounts for a large percentage of the total consumption of bread in Sweden the amount of syrup is often as high as about 20 per cent of the weight of the flour. It therefore seemed of interest to study the loss of added L-lysine HCl under these very unfavourable conditions and also to obtain some information on the protein value of this type of bread in which a mixture of sifted wheat and rye flour is used.[1] The growth rates and nitrogen efficiency ratios of young albino rats were used as measures of protein quality. Besides the animal assay a microbiological method to estimate the loss of added lysine was tried. Liver fat content and liver transaminase activities were also determined.

Experimental

Analytical procedures. The contents of lysine and threonine of the diets were estimated microbiologically. All diet samples were extracted and hydrolysed as described previously (ERICSON *et al* 1961a) before being submitted to the microbiological assays. For the assay of lysine a turbidimetric procedure with *Leuconostoc mesenteroides* P-60 (ATCC 8042) was used. The assay medium was Difco's Bacto Lysine Assay Medium as originally suggested by STEELE *et al* (1949). Instead of incubating the tubes at 37° C for 20 h we preferred 34° C (SCHIAFFINO, MCGUIRE and LOY 1958) for 24 h which gave a straighter standard curve and more reproducible results.

Threonine was also determined turbidimetrically using Difco's Bacto Threonine Assay Medium for *Streptococcus faecalis* (ATCC 8043). The assay tubes were in this case incubated at 37° C for 24 h as recommended by STEELE *et al* (1949).

For further details concerning the microbiological amino acid determinations the reader is referred to ERICSON *et al* (1961a).

The nitrogen content of the ingredients and of the diets was determined by the Kjeldahl method described by PERRIN (1953).

Dry weight determinations were carried out by weighing samples before and after heating at 104—105° C for 24 h. The heated samples were cooled in a desiccator over silica gel.

After the experimental feeding period the animals were killed by decapitation and the livers immediately removed. One part of the liver was cut into small pieces and

[1] The bread will hereafter be referred to as VR bread (Vete = wheat, Rag = rye).

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In a previous paper we reported the loss of added lysine and threonine during the baking of wheat bread (Ericson Larsson and Lid 1961a) It was found that the loss of lysine was rather small — 10—15 % as estimated by animal experiments and 5—10 % as estimated by microbiological assays — whereas the loss of threonine appeared to be considerably greater In a

Amino acids baked into the bread were dissolved in part of the water used for making the dough The L-lysine HCl was a gift from Chas Pfizer & Co Inc (Nasby Park, Sweden) the DL-threonine was purchased from Fluka A. G Switzerland

The diets The diets for the rat experiments were made from 91 % of air-dried and ground bread 3 % of a salt mixture (HEGSTED *et al* 1941) 5 % soya bean oil 0 5 % cod liver oil and a vitamin mixture (HARPER *et al* 1953) The vitamin mixture was first mixed with the salts and then with a small amount of ground bread before it was incorporated in the rest of the diet The quantities of the various vitamins provided by the vitamin mixture and by the cod liver oil were per 100 g of ration thiamin HCl 0 5 mg, riboflavin 0 5 mg niacin 1 0 mg calcium pantothenate 2 0 mg pyridoxine 0 25 mg biotin 0 01 mg pteroylglutamic acid 0 02 mg cyanocobalamin 0 002 mg inositol 10 mg menadione 0 5 mg choline chloride 150 mg vitamin A 375 I U and vitamin D 37 5 I U

Conditions of the rat experiments The rats were males of the Sprague Dawley strain having an initial weight of approximately 50 g when given the experimental diets. Two series of rat experiments were carried out In the first series the rats were housed individually in cylindrical glass containers with a diameter of 20 cm and a floor covering of wood shavings The temperature in the animal room was 27 C In the second series of experiments the rats were kept in wire bottom cages in a room with a temperature of 25 C and a relative humidity of approximately 50 %

The rats were given a basal wheat bread diet for a few days before the experiment began in order to accustom the animals to these types of diets and to the new living conditions It may also have served to standardize the rats with respect to their protein reserves

It should already at this stage be mentioned that the consumption of VR bread by the rats was conspicuously low compared to the consumption of wheat bread

Results

In the first series of experiments which must be considered as of an exploratory nature five groups of rats were given different experimental diets for 35 days Each group consisted of ten rats The first group received the basal wheat bread diet and the second the same diet fortified with 0 44 % L-lysine HCl added before baking[1] The subsequent three groups of rats were fed the basal VR bread diet the basal VR diet fortified with 0 44 % L-lysine HCl of the weight of the wheat flour in the bread added after baking and the basal VR diet fortified with 0 44 % L-lysine HCl of the weight of the wheat flour added before baking

The composition of the diets and the growth rates observed are given in Table I It can be seen that the nonsupplemented VR bread gave a much lower rate of growth than did the nonsupplemented wheat bread This might partly be caused by the lower nitrogen content of the VR diet than of the wheat bread diet However the data can also be taken to indicate a much lower biological value for the proteins of the VR bread It is for instance conceivable that a considerable loss of the protein bound lysine takes place

[1] We have found in experiments with wheat bread that the optimal concentration of L-lysine-HCl is approximately 0 40—0 44 % of the weight of the flour

Table I Composition of diets and gain of weight for the rats used in the introductory series of experiments The contents of lysine and threonine in the diets were not determined Ten rats per group were given the various diets for 35 days

Group	Diet	Nitrogen content¹ %	Growth rate g/day M ± S E
A	Basal wheat bread diet	2 03	1 32 ± 0.10
B	Basal wheat bread diet + 0 44 L-lysine HCl of the flour weight added *before* baking	2 04	4 16 ± 0 19
C	Basal VR bread diet	1 39	0 24 ± 0 06
D	As C but with an amount of L-lysine HCl corresponding to 0 44 of the weight of the wheat flour added *after* baking The amount was increased to 0 44 of the total amount of flour after 24 days	1 46	1 28 ± 0 19 (1 28 ± 0 22)
E	As C, but with an amount of L-lysine HCl corresponding to 0 44 of the weight of the wheat flour added *before* baking	1 46	1 26 ± 0 12

In per cent of the fresh weight of the diet

² This value was obtained during the 24 first days before the level of L-lysine HCl in the diet was increased.

during the baking of this bread due to its high content of reducing sugars Another explanation for the poor growth would be that the rats did not like the taste of the VR diets

A comparison of the growth rates of groups D and E suggests that the loss of added free L-lysine HCl was small in this series of experiments

An attempt was also made to increase the growth rate of the animals in group D by increasing the amount of added L lysine HCl from 0 44 % of the weight of the wheat flour to 0 44 % of the total weight of the flour (wheat + rye) However, this did not result in a faster gain of weight (Table I) which suggested that the protein value of rye flour can not be greatly improved by addition of only lysine

The possibility that the poor growth observed when the rats were given VR diets was due to their distaste of the bread was investigated in a series of experiments in which various additions were made to the VR diet in order to improve its palatability Monosodium glutamate meat extract, a cheese extract water or sodium chloride were tested No drastic effects were observed but there was an indication that a slightly larger consumption occurred in the groups of rats that were given VR diets to which water or salt was added

Table II Composition of diets gain of weight and nitrogen efficiency ratios for the groups of rats in the second series of experiments The diets were given for 22 days

Group	Diet	No of rats	Determined content of			Average gain of weight g	Average nitrogen efficiency ratios g/g
			nitrogen	L-lysine HCl[1]	L-threonine[1]		
A	Basal wheat bread diet	6	2 17	0 48	—	30 1	8 8±0 3
B	Basal wheat bread diet + 0 44 L-lysine HCl	6	2 ?2	0 86	—	101 0	18 2±0 9
C	Basal VR bread diet	9	1 8?	0 34	0 27	5 6 [2](6 4)	2.2±0 3
D	As C, but with an amount of L-lysine HCl corresponding to 0 33 % of the weight of the wheat flour added *after* baking	9	1 90	0 ?0	0.27	44 8	12 6±0 1
E	As C, but with an amount of L-lysine HCl corresponding to 0 44 of the weight of the wheat flour added *after* baking	9	1 89	0 62	0 27	58 0	15 6±0 3
F	As C, but with an amount of L-lysine HCl corresponding to 0 44 of the weight of the wheat flour added *before* baking	9	1 89	0 61	0 27	37 4	11 9±0 6
G	As C but with an amount of L lysine HCl corresponding to 0 475 of the total weight of the flour added *before* baking	9	1 83	0 7?	0.27	52 9	15 0±0 3
H	As C but with amounts of L-lysine HCl and DL-threonine corresponding to 0 ?9 and 0 ?0 of the total weight of the flour added *before* baking	9	1 90	0 81	0 33	83 2	18.2±0 3

[1] In per cent of the dry weight of the diet
[2] After 14 days these rats began to decrease in weight. The figure in parentheses is the gain of weight before this period

Consequently in the subsequent series of experiments 10 % of the diet weights was made up of a solution containing 200 g NaCl per liter

The second series of experiments comprised eight groups of rats The two first groups were given the basal wheat bread diet with and without a lysine supplement corresponding to 0 44 %, L-lysine HCl of the flour weight The

Table III Recovery of added L-lysine HCl from some of the diets of the second series of experiments (see Table II) The contents of L-lysine HCl are expressed in mg per g dry weight of the diet

Diet	Extraction method	Experimentally determined content of added L-lysine HCl mg/g	Theoretical content of added L-lysine HCl mg/g	Recovery
Group D	Twice in cold HCl pH 2.5	1 45	1 47	98 6
	Once in cold NaOH pH 10	1 38	1 47	93 9
Group E	Twice in cold HCl pH 2 5	1 93	1 98	97.5
	Once in cold NaOH pH 10	1 93	1 98	97 5
Group F	Twice in cold HCl pH 2.5	1 43	1 98	72 2
	Once in cold NaOH pH 10	1 46	1 98	73 7

third group received the basal VR diet and the fourth and fifth groups the same basal diet to which 0 33 and 0 44 % L-lysine HCl of the weight of the wheat flour were added after baking The sixth group was given a VR diet containing 0 44 % L-lysine HCl of the weight of wheat flour added before baking and the seventh group a VR diet containing 0 475 % L-lysine HCl of the total amount of the flour (wheat + rye) added before baking The eighth group received a VR diet containing 0 59 % L-lysine HCl and 0 30 % DL-threonine of the total weight of the flour added before baking

In Table II the composition of the diets and the growth rates and nitrogen efficiency ratios[1] for the different experimental groups are shown Again the extremely poor growth of the rats receiving the nonsupplemented VR diet should be noted Also the nitrogen efficiency ratio obtained with the non supplemented VR diet was much lower than the nitrogen efficiency ratio of the nonsupplemented wheat bread diet Supplementation of the VR diet with L-lysine HCl led to a drastic improvement in both growth and efficiency of food utilization However a comparison of the groups D E and F indicates that the loss of supplementary L-lysine HCl during the baking was considerable in this experiment This is also borne out by a comparison of group E and group G

If the growth rates or the nitrogen efficiency ratios observed are plotted against the lysine content of the diets it can be seen that the high growth rate and nitrogen efficiency ratio associated with diet H can not be explained on the basis of the higher lysine content of this diet as compared to diet G but must have resulted from the addition of DL-threonine to diet H The nitrogen efficiency ratio of group H based on VR bread supplemented with

[1] Nitrogen efficiency ratio (NER) = gram gain in weight per gram nitrogen consumed

Table IV *Water and fat content glutamic-oxalacetic acid transaminase (GOT) glutamic pyruvic acid transaminase (GPT) and ornithine-carbamyl transferase (OCT) activities of the livers from the rats of the second series of experiments For composition of the diets given to the different groups see Table II*

Group	Water content	Fat content in per cent of dry weight	GOT[1]	GPT	OCT[1]
			Units per 100 mg wet weight		
A	70 3 ± 0 34	16 6 ± 0 79	2 000	500	1 800
B	70 1 ± 0 23	14 1 ± 0 90	2 500	700	2,200
C	70 7 ± 0 40	14 4 ± 2 55	1 600	300	1,500
D	70 4 ± 0 34	17 3 ± 1 47	1 600	300	1 500
E	71 0 ± 0 46	17 5 ± 1 83	2 300	400	1 700
F	72 2 ± 0 45	16 4 ± 1 15	1 700	500	1 300
G	71 8 ± 0.26	17 5 ± 1 09	1 600	200	2 100
H	72 2 ± 0 21	12 9 ± 0 99	2 800	800	2 300

Only three livers per group were assayed for enzyme activity

both 0 59 % L-lysine HCl and 0 30 % DL-threonine (of the flour weight) was the same as for group B that was based on a wheat bread and supplemented only with 0 44 % L-lysine HCl of the flour weight However it should be kept in mind that the nitrogen content of the VR diets was approximately 15 % lower than that of the wheat bread diets

In a previous paper we tried to estimate the loss of added L-lysine HCl and L-threonine using ordinary microbiological assay methods preceded by acid hydrolysis of the sample (Ericson *et al* 1961a) If the added amino acid does not react with any other component in the bread during the baking it should be possible to recover it by extraction of the bread or the bread diet We have investigated this possibility in a few cases and a full description of the experiments will be given elsewhere When applied to some of the VR diets used in the present investigation the method has given results that agree fairly well with the animal assays This is shown in Table III where the amount of L-lysine HCl that could be extracted with cold dilute hydrochloric acid or alkali are compared with the amounts added to the diets It can be seen that 98—99 % of the L-lysine HCl that was added after baking (groups D and E) could be accounted for in the extracts but that only about 73 % of the L-lysine HCl added before baking occurred in a microbiologically available form (group F) No dilution effects were encountered in the microbiological assay which indicated that the only biologically active material in the extracts was lysine

Table IV shows the remarkable reproducibility of the water and fat contents of the livers from the animals in the different experimental groups

Thus between the groups there are no significant differences with regard to the water and fat contents of the livers The activities of the liver transaminases have a tendency to increase with increasing biological value of the diets, but the number of determinations was too small to permit definite conclusions to be drawn

Discussion

The loss of L lysine HCl during the baking of VR bread can be estimated from the data in Table II by plotting the nitrogen efficiency ratios (NER) as a function of the amount of L-lysine HCl added after baking and the diagram can be used as a standard curve to determine the content of L-lysine HCl (added after baking) that is equivalent to a known amount of L-lysine HCl added before baking If the NER values for groups D E and F are compared in this manner one arrives at a loss of L lysine HCl during baking that is approximately 30 % This is considerably greater than the losses encountered during the baking of wheat bread in which case the loss of L-lysine HCl as judged by a rat growth assay was 10—15 % (Ericson *et al* 1961a) The figure for the VR bread obtained by the animal experiments agrees fairly well with estimates made by extraction of the added lysine followed by microbiological assays (Table III) which indicated a los of 25 %

The data of Table I and II also demonstrate the extremely poor protein value of VR bread It is likely that a great part of the protein bound lysine in the wheat and rye flour used for this bread is destroyed or inactivated during baking due to the high content of reducing sugar in the dough This is particularly unfortunate considering that we here deal with the most widely used type of bread in Sweden One is again by this observation reminded of how important it is that more is learned about the availability of amino acids and other nutrients in food products ready for consumption The finding by Rerinius (1956) that great losses of lysine occurred already during mild heat treatment of a diet simulating that used by the Finnish people should be especially mentioned in this connection

Supplementation of rye flour or rye bread with only lysine does not seem to lead to an increase in protein value comparable to that obtained on lysine supplementation of wheat flour and wheat bread This is indicated by the data in Table I where an increase in lysine above that needed to supplement the wheat flour of the VR bread did not result in an improved rate of growth We have made similar observations also with rye bread and rye flour These studies which will be published shortly indicate that a combination of lysine and threonine is required to assure a substantial improvement of rye protein

The data on liver fat presented in Table IV do not indicate any drastic differences between the groups or any pathological changes of the livers Due to the wide individual variations in the different groups no conclusive

evidence for changes in the GOT GPT- and OCT activities can be given A more complete discussion of these data and of those from a number of other experiments with bread diets will be presented in a separate article (LARSSON RUBARTH and ERICSON 1962)

The authors would like to express their sincere thanks to Mrs. I THOREN Kungsornen AB Stockholm who helped us in many phases of this work, and to Mr P DAHLGREN Kungsornen AB Stockholm whose skill and cooperation during the baking operations are greatly appreciated. Our thanks are due also to Mr E JARVIN AB Westerdahl and Karstens Bakery Stockholm for placing the facilities of the bakery at our disposal and to Mr R. KIHLBERG for experimental assistance

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From the Psychological Laboratory University of Stockholm and The Laboratory of Aviation and Naval Medicine Department of Physiology Karolinska institutet Stockholm, Sweden

Catecholamine Excretion during Mental Work as Modified by Centrally Acting Drugs

By

MARIANNE FRANKENHAEUSER and BIRGITTA POST

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Abstract

FRANKENHAEUSER M and B POST *Catecholamine excretion during mental work as modified by centrally acting drugs* Acta physiol scand 1962 55 74—81 — Experiments were performed to determine (1) whether changes in catecholamine excretion are sensitive indicators of the state of mild stress induced by performing psychological tasks and (2) whether centrally acting drugs would modify such changes Adrenaline excretion was shown to be significantly higher (1) during testing than during inactivity and (2) after intake of metamphetamine (10 mg) than after pentobarbitone (200 mg) No effects on noradrenaline excretion were demonstrated Both drugs induced marked changes in mood but with one exception (verbal performance under pentobarbitone) changes in performance were not found

Excretion of catecholamines has so far received relatively little attention in investigations concerning psychophysiological changes in stress conditions However, the available data indicate that an increased excretion of adrenaline may serve as a sensitive indicator of reactions to stress The significance of noradrenaline in mental stress conditions seems less clearly established

EULER and LUNDBERG (1954) found a significant increase in adrenaline excretion both in pilots during routine training flights and in unexperienced air transported passengers Noradrenaline was also significantly higher during flight than during ground activity in the group of pilots, whereas no change was detected in the group of passengers PEKKARINEN *et al* (1961) found a considerable increase in adrenaline excretion in students undergoing *highly*

stressful examinations but noradrenaline excretion was not much affected ELMADJIAN HOPE and LAMSON (1957) studied the excretion of adrenaline and noradrenaline in hockey players and boxers neuropsychiatric patients and normal subjects in anticipatory states They concluded that active aggressive emotional displays are related to increased excretion of NE (norepinephrine), with or without increased excretion of E (epinephrine) whereas tense anxious but passive emotional displays are related to increased excretion of E in association with normal excretion of NE These conclusions are consistent with the results of COHEN and SILVERMAN (1959) and SILVERMAN and COHEN (1960) which indicate that anger out individuals respond to gravitational stress in a human centrifuge by increased noradrenaline excretion and have high G-tolerance levels whereas anger in individuals react with increased adrenaline and have low G-tolerance levels There is also general agreement between these observations and the adrenaline/noradrenaline relationship in states of fear versus anger suggested by FUNKENSTEIN (1956) In recent experiments on effects of catecholamine infusions by FRANKENHAEUSER JARPE and MATELL (1961) and FRANKENHAEUSER and JARPE (1961) an increase in both adrenaline and noradrenaline excretion was found in a control condition with infusions of Ringer s solution when the subjects performed psychological tasks during the infusions Since however these experiments involved stress factors other than mental work it is not clear whether performing psychological tasks would in itself exert an influence on the excretion of either or both catecholamines

In the present experiments the main questions to be answered were (1) is excretion of catecholamines a sensitive indicator of the state of slight emotional stress induced by performing psychological tasks and if so (2) can changes in mood induced by pentobarbitone and metamphetamine modify these autonomic reactions?

Methods

Subjects

Thirty two medical students 23 males and 9 females participated in the experiments Their ages ranged between 18 and 25 years (average 19 8 years) and their body weights between 48 and 95 kg (average 66 0 kg)

Drug administration

Capsules containing either 200 mg pentobarbitone 10 mg metamphetamine, or a lactose placebo were taken by mouth 1 hour and 15 min before the experiments The reasons for choosing these doses of the drugs have been discussed previously (FRANKENHAEUSER 1959 p 60) All capsules were identical in appearance and neither the subject nor the experimenter knew which drug was being given Before the experiments, which were carried out in the mornings the subjects were allowed a light breakfast (one piece of bread and butter and a glass of milk) The subjects had been told that the capsules contained either a placebo or a small dose of a central stimulant or depressant both of which were in common clinical use

Urinary catecholamines

Before and after each experimental session the subject emptied his bladder by voluntary voiding the urine represented an average secretion time of about 1 hour Immediately after voiding the urinary volume was measured and pH adjusted to 3 5 with HCl Specimens were then stored at + 4 C until analysis which was performed using the fluorimetric technique of EULER and LISHAJKO (1961) Urine was also collected and similarly analysed for about 2 hour periods of relative inactivity during which he subjects were told to read magazines and to refrain from any kind of strenuous ctivity

Subjective ratings

A self rating scale (JONSSON unpublished) consisting of 21 items covering emotional motivational and cognitive reactions was used There are 8 response categories for each item ranging from considerably less than usual to considerably more than usual

Intellectual tests

A factor scale of intellectual ability the F test constructed at the University of Stockholm under the direction of Professor K Harnqvist was used There are two parallel forms each of which consists of four tests one for each factor both parallel forms were used (The procedure of administration as well as the time limits were modified to suit this particular experimental situation)

The verbal factor Opposites Each item consists of a stimulus word and four test words. The task is to identify the test word which is the opposite of the stimulus word There are 40 items arranged in order of increasing difficulty and the time limit was 4 min 0 sec The score was the number of items correctly solved

The numerical factor Multiplication The task is to multiply items consisting of one digit and one 2 digit number There are 50 items arranged in order of increasing difficulty and the time allowed was 30 min 30 sec The score was the number of items correctly multiplied

The inductive factor Letter groups Each item consists of four 4 letter groups Three of these groups are constructed according to a common logical principle whereas the fourth does not follow this principle Example *CFDE* CHIJ KLMN IQRS

The task is to identify the group that differs from the other groups There are 20 items arranged in order of increasing difficulty and the time allowed was 10 min 30 sec The score was the number of items correctly solved

The spatial factor Block counting Each item consists of a drawing of a number of blocks built together The task is to count the number of blocks There are 25 items arranged in order of increasing difficulty and the time limit was 5 min

In order to obtain measures of speed of performance each test was divided into four or five parts depending upon the total number of items in the test each part comprised an equal number of items The subject worked continuously but the time was noted each time he finished one part and thus 4 or 5 time scores were obtained for each test

Stroop s colour word test

Stroop s colour word test in the version described by SMITH and KLEIN (1953) was used In this test the words blue green red and yellow are printed on a card in incongruent colour word combinations There are 100 words and the subject s task is to name the colour of the print ignoring the word as quickly as possible The time was noted after 20 40 60 80 and 100 words Five successive trials were given with 30 sec pauses A scoring procedure is described by SMITH and NYMAN (1959)

General design

The subjects were divided at random into two equally large groups The subjects in *group I* received pentobarbitone in one session and the placebo in the other In *group II* the subjects received metamphetamine and the placebo In both groups the drug and the placebo were given to alternate subjects first or second The procedure was exactly the same in the drug and placebo experiments, except that different parallel forms of the F test were used The F test was given first followed by the rating scale and the colour word test The urine sample was collected at the end of the session

Results

Catecholamine excretion

The results of the catecholamine analyses are shown in Table I The first problem concerns the effects of mental work on catecholamine output The table shows that the adrenaline output in both groups of subjects was higher in the placebo condition when the subjects performed the psychological tests than during the period of inactivity The difference is statistically reliable in both groups the t values based on mean intra pair differences are 2 299 (df = 15 $P < 0 05$) for *group I* and 3 980 (df = 13 $P < 0 01$) for *group II* (Because of a technical mishap catecholamine analyses were obtained for 14 subjects only in *group II*) The differences in noradrenaline excretion between the inactivity and placebo conditions were negligible. Thus the stress induced by performing psychological tasks caused an increase in adrenaline excretion whereas noradrenaline excretion was not affected

The next problem concerns the effects of the two drugs on the catecholamine excretion Table I shows that in *group I* there were no noticeable differences between either adrenaline or noradrenaline output in the placebo and pentobarbitone conditions In *group II* the adrenaline excretion was somewhat higher after intake of metamphetamine than after the placebo but this difference is not statistically reliable ($t = 1 886$ df = 13 $P > 0 05$) Noradrenaline excretion was not affected by the drugs

A comparison of the two groups of subjects shows that the adrenaline values were consistently higher in *group II* during all conditions The significance of the difference in adrenaline excretion between the two groups in each of the three conditions was calculated by means of the t test The adrenaline values for the two identical conditions for *group I* and *group II* (inactivity and placebo conditions) did not differ significantly However the comparison of the two drug conditions showed adrenaline excretion to be significantly higher after intake of metamphetamine than after pentobarbitone ($t = 2 791$ df = 27 $P < 0 01$) Since no significant differences were found between drug and placebo conditions in either group of subjects one possible interpretation of the difference found between the two drug conditions is that metamphetamine and pentobarbitone affect adrenaline excretion in opposite directions

Table I Means and S E for urine volume time for urine secretion for sample and urinary excretion of catecholamines in two groups of subjects

	Group I			Group II		
	Inactivity	Mental work		Inactivity	Mental work	
		Placebo	Pentobarb		Placebo	Metamph
Urine ml	174 ± 32 2	97 ± 23 4	76 ± 16 0	205 ± 37 5	135 ± 28 5	121 ± 25 7
Time for urine secretion min	127 ± 8 7	58 ± 3 6	59 ± 3 6	115 ± 4 3	52 ± 2 0	56 ± 2 5
Excreted adr ng min	6 8 ± 1 3	11 0 ± 1 2	10 8 ± 1 5	8 4 ± 1 9	15 2 ± 2 6	19 3 ± 2 6
Excreted noradr ng min	19 3 ± 2 0	22 4 ± 2 6	21 0 ± 1 9	20 0 ± 2 0	23 9 ± 3 9	22 2 ± 2 1

Subjective reactions

The self ratings under the placebo conditions indicated that the subjects on the whole felt at their ease during the experiments and were not markedly disturbed by the tests given Comparisons were made between subjective ratings under pentobarbitone and placebo conditions and under metamphetamine and placebo conditions As was to be expected the two drugs had on the whole opposite effects on the subjects mental state The changes induced by pentobarbitone were more numerous and more pronounced than those produced by metamphetamine

Statistical analyses of the differences between pentobarbitone and placebo conditions showed that the primary subjective effects of this drug were to reduce feelings of alertness and efficiency Statistically significant differences were obtained in responses to the items *alert tired* ($P < 0\ 001$) *drowsy energetic clear headed working capacity* ($P < 0\ 05$) All differences were in the direction of *decreased* alertness There were no marked changes in such aspects of mood as happy carefree friendly irritated or calm restless

Comparisons of ratings under metamphetamine and placebo conditions showed that this drug induced feelings of increased *efficiency self confidence* and *carefreeness* However the differences were on the whole slight and statistically significant only for two items *tired* and *drowsy* ($P < 0\ 05$) indicating an *increased* alertness There was no indication of a change in feelings of relaxation.

Performance

The results obtained in the F test were analysed with regard to speed and accuracy of performance (Table II) Under pentobarbitone as compared with placebo there was a statistically significant decrease in the number of items

Table II Means and S E for number of items correctly solved in intellectual tasks during drug and placebo conditions in two groups of subjects

Task	Group I		Group II	
	Placebo	Pentobarb.	Placebo	Metamph.
Verbal	36.19 ± 0.60	34.25 ± 0.92	35.75 ± 0.[illegible]0	36.63 ± 1.04
Numerical	42.19 ± 1.11	41.25 ± 1.56	42.94 ± 1.98	42.69 ± 1.86
Inductive	15.44 ± 0.61	15.19 ± 0.67	16.81 ± 0.72	16.69 ± 0.51
Spatial	16.80 ± 0.62	16.63 ± 0.80	16.69 ± 0.57	16.63 ± 0.43

correctly solved in the verbal test ($t = 2.350$ $df = 15$ $P < 0.05$) but no changes in the other tests in either speed or accuracy No effects of metamphetamine on performance were seen

The data obtained in the colour word test did not permit classification according to the principles of SMITH and NYMAN (1959) of a sufficient number of subjects to make possible a statistical analysis of drug effects The data were also treated in a conventional manner by calculating the mean time taken to complete the test under each condition Performance was found to be some what slower under pentobarbitone and quicker under metamphetamine None of these differences however reached a statistically significant level

Comments and Conclusions

The experiments showed (1) that the excretion of adrenaline increased dur ing the performance of psychological tasks whereas noradrenaline excretion was not affected and (2) that adrenaline excretion was significantly higher after intake of metamphetamine than after pentobarbitone whereas the drugs did not influence noradrenaline excretion

No reliable differences in adrenaline excretion were found between placebo and pentobarbitone conditions (*group I*) or placebo and metamphetamine conditions (*group II*) This fact in combination with the clearly demonstrated differences between adrenaline excretion in the two drug conditions suggests that the two drugs act in opposite directions on adrenaline excretion Such an interpretation is consistent with the changes in mood also in opposite direc tion induced by the two drugs

The aspects of mood affected by the drugs were primarily those related to alertness whereas for example items indicating degree of relaxation ("calm restless) were not influenced by either drug That pentobarbitone failed to induce feelings of relaxation may have been due to the increased strain of per forming tasks when feeling tired Hence it seems conceivable that barbiturates

might act more strongly to reduce adrenaline excretion in other types of stress-situations where one is not required to concentrate on doing mental work.

During pentobarbitone the actual performance of the subjects remained undisturbed in three of the four intellectual tests, but the subjects felt that they were working less well. This discrepancy between actual performance and the subject's own impressions of his efficiency suggests that the subjects were able to "pull themselves together" for short periods and overcome deleterious drug effects. It should be pointed out that when the same tests were used in experiments with nitrous oxide (30 % N_2O and 70 % O_2) severe performance decrements were found (FRANKENHAEUSER and BECKMAN 1961).

It may be argued that the increase in adrenaline excretion in the placebo conditions was associated with the intake of the capsules rather than with the testing. Although possible effects of placebo on adrenaline excretion cannot be excluded such an interpretation appears farfetched in view of the fact that catecholamine excretion in a previous investigation (FRANKENHAEUSER, JÄRPE and MATELL 1961) was shown not to be affected by placebo infusions, when the subjects were not engaged in mental activities. Consequently it seems justifiable to regard the psychological testing as the main stressor.

It may also be asked whether the present experimental situation can be considered a mild stress situation. The general attitude of the subjects certainly indicated that they did not consider the situation particularly disturbing. Such an attitude was to be expected, since the subjects were all medical students and were used to frequent examinations. They had also been informed that their test results had no bearing on their academic work and would be treated confidentially. Furthermore the experimenters were students of about the same age as the subjects. For these reasons the situation can be regarded as only mildly stressful and an increase in adrenaline output may thus be considered a sensitive indicator of reactions to mental stress. In order to assess the sensitivity of adrenaline excretion as compared with other physiological processes commonly used as indicators of stress reactions, such as heart rate, blood pressure, skin resistance and muscle tension (see e. g. review by MARTIN 1961) simultaneous measurements of the various reactions would of course be required. In this connection it is interesting to note that in experiments similar to those presented here heart rate was found to increase during mental work (STEINBERG, personal communication).

Thus the present investigation suggests that urinary excretion of adrenaline provides a sensitive measure of reactions to mental stress, and that centrally acting drugs may influence adrenaline excretion during mental work.

We wish to thank Dr. C. O. Jonsson, Leckomberga Hospital, for placing the rating scale constructed by him at our disposal; Mrs. I. Jansson, Mrs. L. Mathiasson and Mr. P. A. Ro, AS, for assistance in the psychological work; and Miss V. Bruno, Department of Physiology, Karolinska institutet, for carrying out the catecholamine analyses. The investigation was supported by a grant from the Swedish Council for Social Science Research.

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We wish to thank Dr C O JONSSON Beckomberga Hospital for placing the rating scale constructed by him at our disposal. Mrs I JANSSON, Mrs L MATHIASSON and Mr P A RÖNNAS for assistance in the psychological work and Miss V BRING Department of Physiology Karolinska institutet for carrying out the catecholamine analyses. The investigation was supported by a grant from the Swedish Council for Social Science Research

In this paper a series of experiments in anesthetized dogs at „steady state" will be presented in which the regional oxygen consumption ($rCMR_{O}$) and carbon dioxide production ($rCMR_{CO}$) of the cerebral cortex was determined and related to the EEG. A new repeatable method for measuring the regional cortical blood flow (rCBF: LASSEN and INGVAR 1961) was used in combination with determinations of the oxygen and carbon dioxide content of arterial and cortical venous blood samples.

The purpose of the present series of experiments was twofold. Firstly we wanted to develop an experimental procedure for studies of the regional metabolism and the regional functional activity of the brain *in vivo*. Secondly quantitative measurements were also made of the tissue oxygen and carbon dioxide tension (tissue pO_2: LUBBERS 1960; tissue pCO_2: SIESJO 1961) in the cerebral cortex together with analyses of the arterial and cortical venous pO_2 and pCO_2. These determinations were carried out to study the relationship between the calculated (THEWS 1960) and the directly measured tissue pO_2 and pCO_2 (GLEICHMANN *et al.* 1962). Preliminary summaries of the present experiments have been presented previously (INGVAR 1961 a and b; LÜBBERS 1960).

Methods

The present results are based upon 9 experiments in dogs of which 8 were successful. Prior to this series 31 experiments were made in 11 dogs, 16 cats and 4 rats in which all the different techniques involved were successively developed and tested.

The dogs (weighing 4.5 to 10 kg) were all anesthetized with Nembutal (30—35 mg/kg) injected into a vein of the fore paw. The Nembutal anesthesia was later renewed during the experiments by doses of about 10—15 mg/kg administered intravenously. Initially 150 000 I. U. of penicillin and 0.25 g dihydrostreptomycin (1 ml of Streptopenin Kabi Stockholm) was administered intramuscularly. The experiments lasted 10—18 hours.

After tracheotomy heparinized polyethylene cannulae were mounted in both femoral arteries for recording blood pressure by means of a capacitive electromanometer (Elema, Stockholm) and for arterial blood sampling. Two further cannulae were mounted in the femoral veins for administration of drugs and fluids. Blood transfusions were sometimes given with blood aquired the same day from another dog. Dextran (Pharmacia Uppsala) was used as a blood substitute. For the determination of cortical blood flow two thinner cannulae were mounted in the central end of the cut lingual arteries. The tips of these two cannulae were brought up into the carotid arteries to end at the carotid bifurcation.

The head of the dog was mounted in a head holder. Craniotomy was then made after resection of both temporal muscles. In order to eliminate extracerebral inlets from diploic anastomoses to the superior sagittal sinus a midline resection of the cranial vault (20 mm in width) was made. Bleeding from the opened diploic veins was stopped with small pieces of Oxycel (PARKE DAVIS and Co). Care was taken not to injure the dura during this procedure. Following this the bony defect was covered with a fasthardening dental cement (Swedon Svedia Co Enköping Sweden). The most occipital part of the craniotomy was however left open for the subsequent cannulation of the superior sagittal sinus (see below).

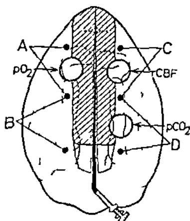


Fig 1 Diagrammatic representation of the experimental set up The head of the experimental animal is seen from above The Geiger Muller tube and the oxygen cathode were placed symmetrically over the posterior sigmoid gyri and the carbon dioxide electrode somewhat posteriorly on one side The EEG electrodes were placed symmetrically over both hemispheres as indicated The hatched area denotes cranioplasty with dental cement after elimination of the diploic anastomoses by longitudinal craniotomy The placement of the polyethylene cannula within the superior sagittal sinus is also indicated

When the cranioplasty had hardened three circular openings in the cranial vault were made for the Geiger Muller tube (see below) the pO_2 and the pCO_2 electrodes respectively (Fig 1) The first two openings mentioned were made symmetrically over the posterior sigmoid gyri The dura was left intact in the three openings 6 EEG electrodes (brass screws) were then mounted in small burr holes symmetrically over the two hemispheres The blood pressure and occasionally the cortical pO_2 and pCO were recorded continuously be means of DC channels of an 8 channel OFFNER Type D3 electroencephalograph Channels used for EEG recording had a linear frequency response between 0 5 and 70 cps A paper speed of 2 5 or 25 mm per second was used

The dog was then transferred to an experimental table and placed upon an electrical heating cushion A thermocouple was placed deep down in the oesophagus and connected to an electrothermometer (Electrolaboratoriet, Copenhagen) Another thermocouple was placed on the cortical surface Between 0 5 and 1 0 ml of Flaxedil (MAY and BAKER) was then given and artificial respiration started (The first three preparations of the series were not curarized but breathed spontaneously, see Table I) Intravenous infusions of blood or Dextran were also made 100 mg of Heparin (Vitrum Stockholm) in 10 ml saline was then given i v

Determination of regional cortical blood flow (rCBF) was made according to the method recently developed by LASSEN and INGVAR (1961) According to this method a small volume (1—4 ml) of a 0 9 per cent saline solution equilibrated with radioactive Krypton gas was injected in 5—20 sec into one of the carotid arteries and the subsequent rapid uptake and slower clearance of the radioisotope was measured on the homolateral cortical surface with a Geiger Muller tube coupled to a rate meter The blood flow was calculated from the clearance curves after correction for inequalities in tissue saturation due to the short injection times used (LASSEN and INGVAR 1962)

After heparinization the superior sagittal sinus was cannulated (cf HOMBURGER et al 1946) with a small polyethylene tube (outer diameter 2 0—2 5 mm inner diameter 1 8—2 3 mm length 5—6 cm) with a slightly tapered tip and with a cut hypodermic needle and a stopper mounted in the other end The cannula was introduced through a small incision in the dorsal wall of the superior sagittal sinus The incision was made so small that the cannula filled it completely and no bleeding occurred around the cannula after its introduction Within the sinus the cannula did not fill the lumen The frontal end of the cannula was brought forward to a point near the entrance of the two large parasagittal veins coming from the sigmoid gyri on either side (Fig 1)

The venous and the arterial samples were drawn slowly from the sinus and the femoral artery respectively with paraffined and heparinized 2 5 ml all glass syringes which had been heated to body temperature The syringes were slowly flushed 2 or 3 times with blood from the vessels before the final sample (2 5 ml) was taken The aspiration period was 3 to 5 min. Samples containing air bubbles were discarded Immediately after the samples had been taken *the syringes were sealed with snugly fitting plastic stoppers and* transferred for gas tension measurements and the manometric analyses of oxygen and carbon dioxide content

The pO_2 and pCO_2 of the blood samples were determined by means of the rapid simultaneous methods developed by GLEICHMANN and LÜBBERS (1960) The error of such determinations is less than 1 % The pH of the samples was determined by means of the micromethod of SIGGAARD ANDERSEN *et al* (1960) The oxygen and carbon dioxide content of the samples was measured in double determinations with the VAN SLYKE method (VAN SLYKE and NEILL 1924) Hemoglobin concentration was determined with a Ljungberg colorimeter

The cortical tissue pO_2 was measured directly on the cortical surface after excision of the dura with the chamber electrode of LÜBBERS (1960) which permits both continuous relative, as well as discontinuous absolute measurements of the tissue pO_2 The cortical tissue pCO_2 was measured similarly with the electrode developed by SIESJO (1961) In anesthesia the placement of the pCO_2 electrode on the cortical surface is not critical (SIESJO 1961) and it was therefore placed about 2 cm posteriorly to the other surface measuring devices (Fig 1) The results of the tissue gas tension measurements will be dealt with in a separate communication (GLEICHMANN *et al* 1962)

Experimental procedure Constancy of temperature blood pressure respiration and EEG was considered a prerequisite for the present steady state experiments For this purpose the anesthesia was often supplemented in order to achieve a preparation with a stable EEG pattern which did not react to the many different measuring procedures Cooling or warming was applied so that the oesophageal temperature of the preparation was kept as close to 38 C as possible The respiratory state was judged by measuring the arterial pCO frequently and the pump was adjusted so that an arterial pCO_2 of about 30—40 mm Hg was maintained Blood transfusions or Dextran were given to stabilize the blood pressure if necessary Once steady state was considered to be present a series of determinations were made Usually rCBF was measured at first After about 3—4 minutes the blood samples were taken often simultaneously with another rCBF determination Finally another rCBF measurement was made again The values of cortical tissue pO_2 and pCO were noted if measured during the time of the blood sampling Notes were also made of the temperature in the oesophagus and on the cortical surface at the sampling time In later experiments the hemoglobin concentration and the hematocrit were also measured at each sampling period In some of the later experiments up to 5 complete sets of determinations could thus be made

Results

The main results are found in Table I There were 26 complete sets of determinations which permitted a calculation of $rCMR_{O}$ and $rCMR_{CO}$ During these determinations the blood flow measurements were either made simultaneously with the blood sampling or 1—5 min before and/or after the sampling In all these cases except one the systemic circulation the respiration and the EEG showed sufficient constancy to permit the conclusion that the rCBF values measured were representative for the rAVDs obtained

Table I 1

Experiment		Artery					Sinus				
		O		CO		pH	O_2		CO_2		pH
		p	Vol	p	Vol.		p	Vol %	p	Vol %	
I		—	14 23	—	40 68	—	—	1 88	—	53 65	—
II		95 0	15 97	26 0	33 64	7 345	19 5	1 92	54 0	49 82	7 230
III	1	96 0	16 34	34 0	30 60	7 360	22 8	3 40	56 5	44 82	7 225
	2	85 0	13 42	34 5	36 46	7 350	23 5	2 69	62 0	47 06	7 220
	3	92 0	13 03	26 5	18 26	7 255	16 5	1 11	49 5	31 32	7 055
IV	1	75 5	16 37	29 0	29 36	7 375	22 0	3 22	51 0	43 83	7 235
	2	65 0	14 77	29 5	29 80	7 360	22 0	2 91	49 5	43 47	7 255
	3	70 0	15 51	27 5	29 46	7 395	24 5	3 29	49 5	43 64	7 250
	4	70 0	14 08	0 0	34 46	7 240	24 0	2 24	59 0	47 42	7 130
V	1	90 5	16 75	30 3	35 81	7 340	32 5	3 92	50 5	48 80	7 280
	2	88 0	17 17	31 5	33 20	7 330	28 5	3 38	53 0	47 54	7 275
	3	80 0	16 19	43 0	36 94	7 285	50 0	4 85	69 0	49 00	7 240
	4	94 5	16 32	22 0	29 03	7 380	19 5	2 91	40 0	42 91	7 330
VI	1	95 0	21 67	22 0	28 49	7 380	23 5	5 46	44 0	44 0	7 370
	2	90 5	22 53	31 0	29 79	7 328	43 5	12 29	48 5	38 67	7 233
	3	75 0	15 37	44 5	35 22	7 250	45 5	9 57	55 0	41 63	7 265
	4	77 5	13 37	40 5	37 60	7 287	44 0	6 71	53 5	44 50	7 260
	5	80 5	11 34	40 5	35 71	7 275	44 5	5 20	55 0	42 35	7 247
VIII	1	91 0	21 94	30 0	34 23	7 280	45 7	10 48	50 0	45 71	7 200
	2	83 0	21 36	30 5	35 51	7 245	44 2	12 50	61 0	44 61	7 180
	3	84 0	21 83	44 5	33 62	7 270	38 5	11 24	54 0	44 74	7 190
	4	81 0	17 94	50 0	37 50	7 205	41 7	9 34	72 5	46 60	7 155
IX	1	98 0	18 32	30 0	42 40	7 435	26 4	9 19	49 5	52 11	7.315
	2	98 0	19 47	29 5	38 94	7 435	27 2	5 77	48 5	52 76	7.235
	3	98 0	19 51	30 0	38 16	7 415	25 3	4 94	51 5	52 52	7 250
	4	113 0	19 69	14 2	97 35	7 620	20 7	5 01	28 0	42 45	2 460
	5	30 5	6 02	52 0	50 48	7 195	19 5	1 38	63 0	50 48	7 150

The exceptional case concerns determination VI 1 (in Table I marked by parentheses) in which the rCBF value given with all probability was too low In this case the blood flow was measured three minutes before the blood sampling was made during a period with typical barbiturate spindles in the EEG When the blood sampling was started presumably due to manipulation with the sinus cannula the dog woke up suddenly and there was a very intense arousal reaction in the EEG which outlasted the sampling period The high values of rAVDo and rAVDco are noteworthy (See discussion)

Table I 2

R Q	AVD		rCBF	rCMR		EEG		
	O	CO		O	CO_2	Dominant frequencies		
1 05	12 35	12 97	0 44	5 4	5 7	2—4	S	some 8 cps
1 15	14 05	16 18	0 37	5 2	6 0	1—3	M	
1 10	12 94	14 22	0 56	7 3	8 0	1	M	some 8 cps
0 99	10 73	10 60	0.57	6 1	6 0	0 5—2	S	fast absent
1 09	11 92	13 06	—	—	—	0 5—2	S	—
1 10	13 12	14 48	0 43	5 6	6 2	2—4	M	some 8 cps
1 15	11 86	13 67	0 34	4 0	4 6	0 5—3	S	less 8 cps
1 16	12 22	11 43	0 45	5.5	5 1	0.5—4	S	—
1 10	11 84	12 96	0 19	2.3	2 5	0 5—3	S	some 10 cps
1 01	12 83	12 99	0 54	6.9	7 0	8—10	F	some 4 cps
1 04	13 79	14 34	0.56	7 7	8 0	3—5	M	some 8—10 cps
1 06	11 34	12 06	0.57	6 5	6 9	7—10	F	very few 2—3
0 98	13 66	13 41	0 49	6 7	6 6	2—3	S	spindles
0 96	16 21	15.51	(0 79)	(12 8)	(12 3)	above 10	F	arousal
0 87	10 24	8 88	0 61	6 3	5 4	3—10	M	variable pattern
1 10	5 80	6 41	0 69	4 0	4 4	4—10	M	some spindles
1 03	6 66	6 90	0 82	5 5	5 7	7—12	F	few spindles
1 08	6 14	6 64	0 89	5 5	5 9	2—10	M	—
1 01	11 46	11 48	0 53	6 1	6 1	6—8	F	some 2 cps
1 03	8 66	9 10	0 62	5.5	5 6	6—8	F	some 2 cps
1 05	10 59	11 12	1 06	11 2	11 8	4—6	M	some 2 cps
1 06	8 60	9 10	1 00	8.6	9 1	4—6	M	less 2 cps
1 06	9 13	9 71	0 78	7 1	7 6	8—10	F	some 2—4 cps
1 01	13 70	13 82	0 71	9 7	9 8	8—10	F	some 2—3 cps
0.99	14 57	14 36	0 83	12 1	11 9	5—7	F	some 2—3 cps
1 03	14 68	15 10	0 91	13 4	13 7	8—10	F	some 4—6 cps
0 76	4 64	4.52	1 05	4 9	3 7	2—3	S	a few 8 cps

In the first 10 columns of Table I the determinations of blood oxygen and carbon dioxide tension and content as well as of pH are given These values reflect the state of oxygenation and respiration of the different preparations There were some high pH values obtained when slight hyperventilation was carried out and some low values during hypoventilation Some of the deter minations were deliberately made *sub finem* shortly before the animal died In most of these cases there were signs of brain edema anemia and/or asphyxia a fact which might explain the aberrant values recorded These values are in

Table 1 3

Class	Temp C		Hb,	BI	Remarks
	Oes	Cortex			
slow	40 0	—	—	140/80	
mixed	38 1	–	—	110/70	Falling blood pressure
mixed	37 9	—	—	150/100	Respiration changed
slow	38 4	—	—	130/105	—
slow	38 2	—	—	80/50	Moribund
mixed	38 2	38 4	14 2	150/80	—
slow	38 6	38 7	—	150/90	—
slow	38 9	39 0	13 7	150/80	—
slow	39 6	39 2	—	140/80	Moribund
fast	38 2	37 8	—	150/90	—
mixed	38 8	37 7	14 8	140/100	—
fast	38 7	37 5	—	150/100	—
slow	36 5	36 4	13 4	130/90	Moribund
fast	38 4	38 0	17 9	110/80	Marked arousal
mixed	39 2	9 3	19 9	150/110	—
mixed	39 3	39 4	13 8	140/100	Bleeding
fast	38 9	39 1	11 4	130/90	Bleeding
mixed	38 8	38 5?	8 5	100/70	Moribund
fast	38 6	38 3	17 6	145/90	—
fast	38 9	38 8	17 6	150/100	—
mixed	38 6	38 5	17 6	140/95	—
mixed	39 1	39 0	15 3	135/80	Brain edema hypoventilation
fast	38 2	37 0	14 2	125/100	—
fast	38 4	37 5	14 9	130/95	—
fast	38 7	37 8	15 0	115/85	—
fast	38 2	37 5	14 3	90/70	Marked hyperventilation
slow	38 0	37 8	15 9	130/80	Hypoventilation moribund

general excluded from the following discussion Some of the variables determined will now be briefly commented upon

The regional cortical respiratory quotient RQ From our present knowledge of the metabolism of the brain it should be expected that the regional cortical RQ should also be close to unity (Himwich 1951 McIlwain 1959) As seen in the corresponding column of Table I this was found to be the case in the majority of the determinations (*cf* Schmidt Kety and Pennes 1945)

The regional cortical blood flow rCBF A mean value of all 26 determinations

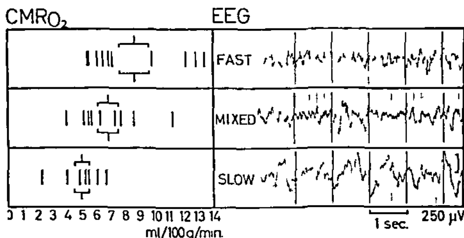


Fig 2 Correlation between regional cortical oxygen uptake rate and EEG The EEG pattern prevailing in all leads at the time of the metabolic determinations as classified as 1 *Fast* when dominated by frequencies of 5 to 12 per second and slow waves were essentially absent as 2 *Mixed* when both such fast waves as well as low waves were present and as 3 *Slow* when high voltage waves of 0 5 to 4 cycles dominated the pattern Each vertical bar denotes one determination of rCMRo The mean and standard error of mean are also included for each class On the right side of the diagram three typical samples are shown

of rCBF in the sensorimotor area (postsigmoid gyrus) of the dog gave a flow of 0 65 ml/g/min standard error of mean (SE) ± 0 05 This figure represents different depths of anesthesia and different states of functional activity Admittedly such an average value is of very limited interest It merits mentioning however since it agrees very well with the one reported by SOKOLOFF and his coworkers (SOKOLOFF 1961) for the regional blood flow in the sensorimotor cortex of the cat during light Pentothal anesthesia (0 65 ml/g/min ± S E 0 07) Their determinations were made with the decapitation method of KETY *et al* (1955) In non anesthetized cats the same cortical area showed a blood flow of 1 38 ml/g/min (± S E 0 12)

The arterio-venous differences for oxygen and carbon dioxide $rAVD_{O}$ *and* $rAVD_{CO}$

The majority of the rAVDs were found in the range of 10 0 to 14 0 vol per cent (averages 11 3 ± S D 2 9 and 11 7 ± S D 3 1) a fact indicating a fair constancy of these variables This confirms previous studies of the brain as a whole by NOELL and SCHNEIDER (1934) and KETY and SCHMIDT (1945) who also demonstrated a constancy of the cerebral a v difference of oxygen in nonanemic normoventilated organisms The values found are in general higher than those found for the whole brain in monkeys by SCHMIDT *et al* (1945) but they agree with those reported by HOMBURGER *et al* (1946) from studies of sinus blood in the dog (5 determinations range 5 4 to 12 8 average 10 0) There were in the present series some rAVDs lower than 9 0 vol per cent in which anemia (VI 4 and 5) and/or asphyxia (VI 3 VIII 4 and IX 5) might have been of importance

comparison to the non anesthetized state only smaller differences in regional cortical blood flow are seen in the anesthetized state (SOKOLOFF 1961) In summary, there is therefore reason to believe that at steady state the samples obtained in the superior sagittal sinus really were representative for the smaller area in which the rCBF determinations were made It seems necessary however here to make a reservation for certain non steady state conditions (see below)

It should be mentioned that in the present series of experiments we have not taken full advantage of the true regional nature of the technique used of measuring the blood flow rate This would have required a blood sampling from a superficial cortical vein coming from the area (about 35 square mm) on which the Kr^{85} measurements were made There are obvious risks for the regional circulation and metabolism involved in the cannulation of the superficial cortical vessel and since the aim of the present work only was to take gross general changes of the cortical functional activity into consideration, we confined ourselves to the study of cortical venous samples from the superior sagittal sinus

Correlation between cortical functional activity and cortical oxygen uptake rate The crude correlation between EEG pattern and $rCMR_0$ found in the present experiments as shown in Fig 2 confirms observations from large series in man It has repeatedly been demonstrated that in the comatous state irrespective of its cause there is a marked reduction of the total oxygen uptake of the human (reduction from 3 3 to about 2 0 ml/100 g/min *cf* summary by LASSEN 1959) In such patients the EEG shows a slow wave pattern with high voltage delta waves (0 5 p 3 cps) We interpret the group of observations in which a slow EEG pattern was found as due to deep anesthesia and/or asphyxia and/or traumatic factors pertaining to the experiment It is therefore reasonable to exclude this group from a consideration of what could constitute the normal oxygen uptake rate of the cerebral cortex

An average $rCMR_0$ for the cerebral cortex generating a mixed or fast wave EEG pattern was found to be 7 7 ml/100 g/min in average in the present series a figure which compares favourably with that of HOMBURGER *et al* (1946), mentioned above Since our experiments were carried out in barbiturate anesthesia it seems reasonable to conclude that the oxygen uptake of the cerebral cortex under normal physiological condition is higher However the effect of painful stimuli and anxiety causing arousal should not be overlooked, since there are several reports in the literature that such factors might be associated with cerebral oxygen uptake rates which are substantially higher than at rest (SCHMIDT *et al* 1945 SOKOLOFF 1956 KETY 1961 LASSEN LEINBERG and LANE 1960) In this connection it is of interest to note regarding especially the four high values of $rCMR_0$ found here that SOKOLOFF (1961) in ten conscious cats for the sensory motor cortex found an $rCBF_0$ of 1 38 ml/g/min Using an $rAVD_0$ value obtained in the present experiments as well as by HOMBURGER *et al* (1946) of about 10 5 vol % the $rCMR_0$ for the same

area in the conscious cat calculates to 14 5 ml/100 g/min We cannot avoid the impression that this very high value could have been due to the above mentioned factors *e g anxiety and arousal present in the restrained conditions of their* experimental animals Such states obviously differ from the physiological resting state and any conclusion as to their inherent cortical metabolism should be made with extreme caution (CLEICHMANN *et al* 1962) More experiments are clearly needed in order to determine the oxygen uptake rate of the normally functioning cerebral cortex

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From the Department of Pharmacology University of Goteborg Sweden

Formation of Phenolic Acids in Brain after Administration of 3,4-Dihydroxyphenylalanine

By

Arvid Carlsson and Nils-Åke Hillarp

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Abstract

Carlsson A and N Å Hillarp *Formation of phenolic acids in brain after administration of 3 4-dihydroxyphenylalanine* Acta physiol scand 1962 55 95—100 — When L-DOPA (100 mg/kg body wt) was given i v to rabbits large amounts of first 3 4-dihydroxyphenyl acetic acid (DOPAC) and then homovanillic acid rapidly accumulated in the brain stem The data suggest that the dopamine formed in the brain from the administered DOPA first reaches sites of monoamine oxidase The DOPAC thus formed then reaches sites of catechol O methyl transferase The data also suggest that the accumulated phenolic acids do not easily escape from the brain to the blood

O methylation is a major pathway for inactivation of catecholamines in the body (*cf* Axelrod 1960 Schaepdryver and Kirshner 1961) The presence of 3 4 dihydroxyphenylacetic acid (DOPAC) in the nervous system (Euler 1958 Rosengren 1960) suggests however that in this tissue a direct oxidative deamination also may be important for the metabolism of monoamines In the present work the formation of DOPAC and homovanillic acid (HVA) in the rabbit brain after administration of 3 4-dihydroxyphenylalanine (DOPA) has been studied in order to get further knowledge of the role of the catechol O-methyl transferase and monoamine oxidase (MAO) in the brain

Material and Methods

L-DOPA (100 mg/kg body wt i v) was given to rabbits (2 to 3 kg) After varying periods of time the animals were killed by an i v injection of air The brain was immediately removed chilled with ice and dissected Pons and medulla oblongata were removed from the brain stem. The tissue was homogenized in cold 0 4 N perchloric acid (PCA, 5 ml/g) Ethylenediaminetetraacetic acid (EDTA, 1 mg/ml) was added

to the centrifuged extract which was then neutralized to pH 6 with potassium carbonate at 0°. To remove interfering material the extract was first shaken with ethyl acetate and then passed through a small Dowex 50 column (pH 6). An aliquot of the effluent was brought to pH < 2 with HCl and saturated with NaCl. The phenolic acids were extracted with ethyl acetate which was evaporated in a rotating evaporator containing 5 ml of water. DOPAC was determined spectrophotofluorimetrically after condensation with ethylenediamine (ROSENGREN 1960). The recovery of DOPAC added to brain tissue (5 μg/8 g) was 91 to 105 %.

Another aliquot of the effluent was brought to pH < 2 with metaphosphoric acid and extracted with ethyl acetate. After evaporation to a small volume the extract was chromatographed on paper (benzene — propionic acid — water 20 : 14 : 1, *n* butanol — N HCl 4 : 1). The phenolic acids were visualized by spraying with diazotized p-nitroaniline (STUDNITZ and HANSON 1959).

For determination of DOPA a portion of the PCA extract was brought to pH about 2.5 with potassium carbonate at 0° and transferred to a small cation exchange column (Dowex 50W X4, 50 × 4 mm, treated with 20 ml of 0.5 M potassium phosphate pH 6.5 with 0.5 mg/ml of EDTA). After washing of the column with water DOPA was eluted — free from catecholamines — with 6 ml of 0.1 M potassium phosphate pH 6.5 containing EDTA (0.5 mg/ml). The effluent was collected at 0° and immediately used for determination of DOPA with the same method as that for DOPAC. The condensation product has an intense fluorescence with an activation peak at 360 mμ and a fluorescence peak at 460 mμ (uncorrected). Recovery of DOPA added to brain tissue was 85 to 96.

Results and Discussion

In the brain from normal rabbits the content of DOPA, DOPAC and HVA is too low (Table I and II) to permit determination with the methods used. After administration of DOPA, however, large amounts of these acids appeared. On paper chromatography of extracts containing more than 10 μg each of DOPAC and HVA, no other phenolic acids were detected. The amounts of DOPAC estimated from the chromatograms agreed well with those determined fluorimetrically, and the fluorescence spectra were identical with those of pure DOPAC condensed with ethylenediamine. There is thus little doubt that the methods used are fairly reliable for the purpose of this study. HVA was only estimated semiquantitatively on the chromatograms. The identity of this phenolic acid seems to be established from the time relationship of its appearance in the brain and blood (see below), its chromatographic behaviour and characteristic colour on spraying with diazotized p-nitroaniline, the extraction procedure and the fact that it did not form a fluorescent condensation product with ethylenediamine.

DOPA rapidly appeared and disappeared in the brain and blood after an i.v. injection (Table I). The data suggest that this amino acid — like tyrosine (CHIRIGOS, GREENGARD and UDENFRIEND 1960) — rapidly equilibrates between blood and brain tissue.

After administration of DOPA, DOPAC appears especially in those parts of the brain which normally have a rapid formation of catecholamines in

Table I DOPA (μg/g) in brain and blood of rabbits after i v administration of L-DOPA (100 mg/kg) or DOPAC (100 mg/kg divided in two injections with an interval of 30 min)

Reserpine see Table II

	Time hrs.	Number of animals	Brain stem	Cerebral hemispheres	Blood
Controls	—	2	< 0 2	< 0.2	< 0 2
DOPAC	1	2	< 0 2	< 0 2	—
DOPA	0 5	2	19	28	21
DOPA	1	2	4.3	6 9	4 4
DOPA + Reserpine	1	2	1 9	2 6	2 0
DOPA	2	2	1 4	_5	2.2
DOPA	2	1	1 1	2 0	1 7
DOPA	4	2	< 0 2	< 0 2	< 0.2

Table II DOPAC (μg/g) in brain blood and heart of rabbits after i v administration of L-DOPA (100 mg/kg) or DOPAC (100 mg/kg divided in two injections with an interval of 30 min) Nialamide (100 mg/kg) and reserpine (5 mg/kg) were given i v 20 hrs before the DOPA injection in two experiments

	Time hrs	Number of animals	Brain stem	Cerebral hemispheres	Cerebellum	Blood	Heart
Controls	—	3	< 0.2	< 0.2	—	< 0.2	—
DOPAC	1	2	0 8	0 4	—	11	—
DOPAC	1	1	1.2	0 6	—	12	4 6
DOPA	0 5	2	17	5 0	—	40	—
DOPA	0 5	1	15	4 4	—	36	21
DOPA + Nialamide	0 5	2	0 6	0 3	—	1 5	—
DOPA	1	2	8 6	2 0	1 5	7 3	—
DOPA	1	1	8 1	2.2	0 8	12	8 0
DOPA	1	2	6 4	—	—	3 9	—
DOPA + Reserpine	1	2	7 5	1 9	0.5	5 0	—
DOPA	2	2	7.2	0 8	—	0 9	—
DOPA	2	1	5 7	0.8	—	0 8	< 1
DOPA	2	1	4 8	—	—	—	< 1
DOPA	4	2	1 7	0 3	—	0 5	—
DOPA	4	1	1 5	0 3	—	0 3	—
DOPA	6	2	0 9	< 0.2	—	< 0.2	—

dicating that the acid found is formed in the tissue itself (ROSENGREN 1960) Confirming this much higher concentrations of DOPAC appeared in the brain stem (including the basal ganglia) than in the cerebral hemispheres and cerebellum (Table II) That the phenolic acid did not accumulate through uptake

from the blood is furthermore seen from the data in Table II When DOPAC — instead of DOPA — was administered, high concentrations were found in the blood but only low in the brain, indicating that DOPAC — like p hydroxy phenylacetic acid (CHIRIGOS, GREENGARD and UDENFRIEND 1960) — does not penetrate easily — if at all — into the brain The red blood cells have also a low permeability since most — if not all — of the DOPAC in the blood 1 hr after a DOPA injection was present in the plasma (data not shown)

There is good evidence that the DOPAC found in the brain after DOPA administration comes from oxidative deamination of dopamine formed through decarboxylation of DOPA (ROSENGREN 1960) Other pathways — through transaminations (*cf* SHAW, MCMILLAN and ARMSTRONG 1957, POGRUND DRELL and CLARK 1961) — cannot be excluded but probably are of minor importance from a quantitative point of view (POGRUND DRELL and CLARK 1961) No 3 4-dihydroxyphenylpyruvic acid was detected on paper chromatography but — if present — may have been destroyed on processing the extracts It is note worthy that two inhibitors of monoamine oxidase (MAO) — iproniazid (ROSENGREN 1960) and nialamide (Table II) — prevent the formation of DOPAC from administered DOPA

Of considerable interest is the time relationship of the appearance and dis appearance of DOPAC and HVA in the brain Half an hour after DOPA ad ministration the concentration of the 3 O methylated acid was far below that of DOPAC At 1 hr they were about equal while at 2 and especially 4 hr HVA was the dominating metabolite It thus seems that the dopamine endogeneously formed from DOPA first and rapidly reaches sites of MAO where DOPAC is formed and then DOPAC reaches sites of catechol O methyl transferase This indicates that the MAO may play a fundamental role in the metabolism of monoamines in the brain The finding that MAO inhibitors cause a rapid accumulation of catecholamines in the brain (*cf* CARLSSON LINDQVIST and MAGNUSSON 1960) now gets further significance

It is also of interest that both DOPAC and HVA disappeared more slowly from the brain than from the blood and heart in spite of the fact that the concentration of DOPA decreased rapidly The data suggest that the ac cumulated phenolic acids do not easily escape from the brain to the blood — just as the reverse was found to be true Both DOPAC and HVA disap peared from the blood at about the same rate

The formation of DOPAC and HVA from administered DOPA was not obviously changed in reserpinized rabbits (Table II) Since it is of importance to know whether reserpine interferes with the formation of catecholamines the content of DOPAC in corpus striatum was determined essentially according to the method of ROSENGREN (1960) which — if certain precautions are taken — gives quantitative recoveries Confirming the results of this investigator the concentration was found to be the same (0 21 to 0 26 μg/g) in both normal and reserpinized (5 mg/kg 20 hr) rabbits

In two rabbits (DOPA 1 hr) the hypothalamus and corpus striatum were carefully homogenized in 0.3 M sucrose and centrifuged (*cf* BERTLER, HILLARP and ROSENGREN 1960). Most of the DOPAC (80 %) was recovered in the low speed supernatant. After centrifugation at 145 000 × *g* for 1 hr practically the whole amount (> 90 %) was found in the clear supernatant. HVA showed the same distribution. In contrast to the catecholamines the phenolic acids thus do not seem to accumulate in granules which can be isolated. EULER and LISHAJKO (1961) have shown that DOPAC present in the splenic nerves is not granule bound.

It is obviously important to know the nature of the cells which are capable of forming dopamine from administered DOPA. The distribution of monoamines, DOPAC and dopadecarboxylase in the brain seem roughly to correspond with that of excess dopamine and DOPAC after administration of DOPA (BERTLER and ROSENGREN 1959, ROSENGREN 1960). This indicates that the amine is formed in cells which normally produce and release monoamines. Although more direct evidence would be desirable this assumption seems reasonable. On this basis the localization and function of MAO and methyl transferase may be considered. The time course of the accumulation of monoamines and their 3-O methylated metabolites after inhibition of the MAO in the brain (CARLSSON, LINDQVIST and MAGNUSSON 1960) favours the view that the MAO is close to the site of synthesis and that the methyl transferase is localized outside the amine producing cell and thus attacks amines first when they have been released (CARLSSON 1960). The results obtained in this study — a very rapid accumulation of first DOPAC and then HVA after DOPA administration — further support this view. Both sets of data are easily understood if the MAO here considered is localized *within* the amine producing cell and the O methyl transferase outside it in the vicinity of supposed receptors. This MAO may thus be of primary importance for the regulation of amine formation and the transferase for the inactivation of amines released to the receptors. Further support has recently been found in experiments with the adrenal medulla (made together with C. Hall, unpublished) which seem to *show that MAO — but not O-methyl transferase — is present in the amine* producing cell itself.

If the view briefly outlined above — although oversimplified — is correct *many other data fall in line. It explains for instance 1)* why inhibitors of O methyl transferase — in contrast to MAO inhibitors — potentiate the effect of injected or released catechol amines, 2) why 3-O methylation — and not oxidative deamination — is the principal pathway for inactivation of injected or released catecholamines and 3) why pyrogallol — in contrast to MAO-inhibitors — does not cause an accumulation of catecholamines in the brain and heart (*see* AXELROD 1960, CROUT, CREVELING and CATON 1960, CROUT, CREVELING and UDENFRIEND 1961, HERTTING and AXELROD 1961).

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From the Institute of Physiology, Uppsala University, Uppsala, Sweden

Quantitative Determination of Uveal Blood Flow in Rabbits

By

ANDERS BILL

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Abstract

BILL, A. *Quantitative determination of uveal blood flow in rabbits.* Acta physiol. scand. 1962. 55. 101—110. — In rabbits the uvea is drained essentially by four vortex veins which are nearly equal in size. The veins communicate within the eye through large anastomoses. Investigations were carried out to discover whether the blood flow through the uvea may be determined as the flow from one opened vortex vein multiplied by the number of veins. The results indicate that such a procedure is adequate at intraocular pressures of 10—15 mm Hg or more and normal or low extraocular vortex venous pressures and uveal blood flow rates. It appears that the adequacy of the procedure is due to the fact that under such conditions the pressure in the proximal part of the uveal veins is little different from the intraocular pressure. It also appears that the resistance in the veins draining the uvea may be caused in part by collapse of the vessels provided the pressure and flow requirements mentioned are fulfilled.

The arteries supplying the uvea in various mammals such as humans, dogs and rabbits are relatively small and some of them are very inaccessible. There are two systems of veins, the vortex veins and the anterior ciliary veins. (For anatomical details see LEBER 1903, VILSTRUP 1952.) In all the species mentioned some of the veins are small and, like the arteries, do not permit quantitative flow determinations with any available technique.

As the flow through all the vessels to or from the uvea could not be measured in any species KANEKO (1925) adapted a rather questionable procedure for determinations of uveal blood flow in dogs. One of the vortex veins was opened and the flow from it was determined. KANEKO found the vortex veins

to be so nearly equal in size that it seemed justifiable to determine the total uveal blood flow as the flow from the opened vein multiplied by the number of vortex veins The flow through the anterior ciliary veins was neglected The same procedure was used by many investigators working with rabbits (FISCHER 1930, SONDERMANN 1932 MEESMANN 1930, 1932, LINNÉR 1952 POLLACK and BECKER 1961)

The results obtained were very variable KANEKO found a flow of 0 00157 g/min from one vein in dogs, while in rabbits the mean flow from one vein has been reported to be from 0 236 g/min (FISCHER 1930) to 0 448 g/min (LINNER 1952)

The adequacy of the procedure described was, obviously very much dependent upon whether a vortex vein drains the same amount of blood after it has been opened as before This could be very much doubted, as the veins within the different parts of the uvea, as well as the capillaries, anastomose freely with each other In dogs, the veins draining the uvea anastomose also within the sclera In fact the results reported by KANEKO strongly suggested that this procedure could not be used in dogs The very low values found were presumably due to shunting of blood from the area normally drained by the opened vessel to intact vessels

The purpose of the present work was to determine the adequacy of KANEKO's procedure for rabbits In a previous work (BILL 1962 a) it was found that the blood flow through the anterior ciliary veins in this species is less than 1 per cent of the total blood drainage from the uvea The assumption concerning the dominance of the vortex veins in the drainage of the uvea was thus justifiable It then had to be determined whether the flow through a vortex vein was the same after the vessel had been opened as before and whether the flow through all the four veins was the same

In rabbits there are two superior and two inferior vortex veins One of the veins in each pair is placed nasally and one temporally

The normal intraocular pressure *IOP* in rabbits was reported by WISTRAND (1959) to be 21 6 mm Hg Earlier investigators have reported values from 19 to 38 mm Hg (For references see DAVSON 1956 and WISTRAND 1959)

Methods

Male and female albino rabbits weighing 1 9—3 0 kg were employed

Anaesthesia was induced and maintained with a barbiturate given intravenously (Veterinary Nembutal Abbott initial dose 0 75 ml/kg body weight) The animals were tracheostomized and placed horizontally on one side

The mean arterial blood pressure in a femoral artery was measured with a strain gauge transducer having a moderate volume displacement

In experiments in which there was a blood loss from the eye this was compensated for by intravenous blood infusion

The IOP was regulated artificially in all experiments The anterior chamber was cannulated with a stainless steel cannula connected to a levelling manometer filled

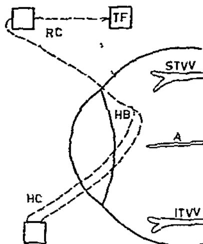


Fig 1 Schematic presentation of the set up used for indirect determinations of changes in uveal blood flow
HC heating circuit *RC* recording circuit *TF* thermos flask *STVV* the superior temporal vortex vein *ITVV* the inferior temporal vortex vein *A* the temporal long posterior ciliary artery In the circuits repre ent constantan wi e copper wire *HB* within the heating circuit represents the part of the constantan wire intermittently heated

with isotonic saline The dimensions of the cannula and the tubings were such as to permit a flow of 0 03—0 05 ml isotonic saline per minute for a pressure difference of 1 cm of water There was no leakage at the cannula As there was sometimes flow in the cannula the height of the levelling manometer did not always give an exact measure of IOP When accurate determinations of IOP were of interest a strain gauge transducer (Elema EMT 456 volume displacement 0 3 mm³/100 mm Hg) was connected to the *communication between the anterior chamber and the levelling manometer* In determinations of the IOP the levelling manometer was disconnected for a moment from the eye by a stopcock.

Heparin (1 500 U/kg body weight) was given i v to prevent coagulation of blood and aqueous humour

The procedure used for indirect determinations of changes in uveal blood flow was a modification of the heated thermocouple principle of GIBBS (1933) The application of this principle in experiments involving the eye was described elsewhere (BILL 1962 b) A small piece of constantan wire is attached to the exposed sclera see Fig 1 The wire is heated intermittently with a constant current and a thermocouple measures *the temperature of the wire When the wire is not heated its temperature* t_U *is higher* the larger the uveal blood flow When it is heated its temperature increases with Δt to t_H The greater the flow through the uvea the smaller is Δt. In the present experiments, changes in blood flow through the retinal vessels and the vessels supplying the sclera were assumed to have no influence on the temperature of the constantan wire

Graphic recorders (Varian G 10) were used for pressure and temperature recordings.

Experiments

A *The effect on the flow of opening a vortex vein*

Procedure The eyelids and the conjunctiva over the superior and temporal parts of the eye were removed and the muscles inserted there were cut close to the eye. The superior temporal vortex vein was isolated and the heated part of the probe was *attached with a fine suture to the part of the sclera situated between the temporal* long posterior ciliary artery and the isolated vein as shown in Fig 1 The part of the

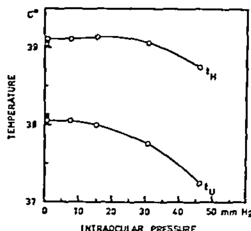


Fig 2 The effect of increasing the intraocular pressure on the temperature of the constantan wire, when heated t_H and unheated t_U and the effect of opening a vortex vein The unfilled square represents the temperature of the wire when heated after opening the draining vortex vein at an intraocular pressure of 0 mm Hg The filled square represents the temperature of the wire when unheated under the same conditions.

uvea which then influenced the probe was drained by the isolated vein. The heated part of the probe was covered with an inert isolating ointment (Silon© Pharmacia) and the reference junction was thermostated outside the eye in a thermos flask. The anterior chamber was cannulated and connected with the levelling manometer

The IOP was decreased stepwise from 45—80 mm Hg to zero with the levelling manometer Each step was 5—20 mm Hg At each level of IOP t_U and Δt were determined This procedure was performed to check that the probe really reacted to moderate changes in flow The IOP was then regulated to different levels in different experiments and t_U and Δt at these levels were determined again. Then the isolated vein was opened The blood was permitted to flow freely over the superior limit of the orbit and t_U and Δt were again determined.

Results In all 18 experiments were performed and 10 were acceptable from a technical point of view In 3 of these the vortex vein was opened at zero IOP in 2 at 10 mm Hg in another 2 at 15 mm Hg and in 3 experiments at 30 60 and 80 mm Hg The mean arterial blood pressure which was stable in each experiment was 75 to 100 mm Hg

When the IOP was decreased before the vein was opened t_U increased and Δt decreased as long as the pressure was not lowered below 10 mm Hg A reduction from this level however to zero produced no changes in either t_U or Δt Opening of a vortex vein gave some increase in t_U and a reduction of Δt in the three experiments performed at zero IOP No such changes could be discovered when the vortex vein was opened at IOPs of 10 mm Hg or more. The result of one of the experiments in which the vein was opened at zero IOP is demonstrated in Fig 2

The changes in t_U and Δt produced by reducing the IOP before the vortex vein was opened indicate that the probe was able to detect changes in local uveal blood flow of less than 10 per cent This could be deduced from the present findings and a study of the relationship between uveal blood flow and IOP reported elsewhere (BILL 1962 c)

A reduction in IOP from 10 mm Hg to zero can be presumed (see discussion) to give very little change in uveal venous pressure and consequently also in uveal blood flow The lack in changes in t_U and Δt found under such conditions are in agreement with this idea

The changes in t_U and Δt produced when the vortex vein was opened at zero IOP indicate that this procedure increased the flow in the territory drained by the vein This in turn indicates that the flow through the vein increased

The absence of changes in t_U and Δt when the vein was opened at IOPs of 10—15 mm Hg or more indicates that under such conditions the opening of the vein did not essentially affect the flow in the area drained by it The probe could not detect changes in direction of flow but there is no reason why the opening of the vein should produce an unchanged flow with an altered direction Thus when a vortex vein was opened at IOPs of 10—15 mm Hg or more the flow through it remained essentially unchanged

B *The role played by each vortex vein in the drainage of the uvea*

Procedure Difficulties in determining the flow from more than two veins at the same time made it necessary to make three series of experiments

In all experiments the eyelids and the upper fibrous margin of the orbit were removed and the lacrimal duct was ligated The head of the animal was placed in such a position that the blood running out of an opened vortex vein flowed over the upper part of the orbit where it formed drops These were collected in test tubes which were weighed before and after the sampling

Each flow determination was based on a group of 2—5 single determinations of the flow during one minute

In one series the flow from the superior temporal vortex vein was compared with the flow from both the superior veins The conjunctiva was removed over the upper part of the eye and the superior rectus and obliquus muscles were cut. The superior temporal vortex vein was then ligated and opened immediately afterwards and the flow from the vein was determined The nasal superior vortex vein was then ligated and opened and the flow from the two opened veins was estimated

It was necessary to rotate the eye somewhat downward to obtain a free flow from the vortex veins Care was taken that the veins were not stretched or kinked

In the other two series the flow through the temporal and inferior pairs of veins respectively was similarly compared

Results In all 32 experiments were performed Of these 23 were performed at IOPs stabilized at levels between 15 to 50 mm Hg while 9 were performed at zero IOP The mean arterial blood pressure was maintained steady at levels between 70 to 110 mm Hg

The error of each single blood flow determination was defined as the standard deviation within the groups of single determinations (DAVIES 1949) In determinations of flow from one vein the error was $\pm$ 0 041 g/min (70 degrees of freedom) and in determinations of the flow from two veins $\pm$ 0 078 g/min (60 degrees of freedom) The error at low flow rates was not different from that at high rates The errors determined include variations in blood flow

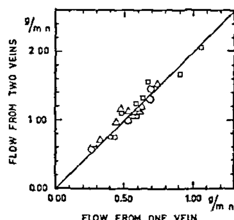


Fig 3 The relationship between the blood flow from two opened vortex veins and that from one Squares represent flow from superior vortex veins, triangles flow from temporal vortex veins circles flow from inferior vortex veins The line represents the relationship expected if in each pair the flow through the different veins had been the same.

caused by slight fluctuations in blood pressure during the sampling and errors connected with the sampling procedure Experiments performed at zero IOP were not included in the error determinations

Results obtained in the 23 experiments performed at an IOP of 15 mm Hg or more are shown in Fig 3 Table I presents the ratio (flow from two opened veins) / (flow from one opened vein) in these experiments The figure and table fail to indicate any systematic differences in flow between the different vortex veins

In the 9 experiments made at zero IOP the ratio was 1 60 ± 0 07 This figure is significantly different from 2 00 ($P < 0.001$) This indicates that at very low IOPs an opened vein drained some of the blood normally drained by still unopened veins

Table I The ratio (flow from two opened veins) / (flow from one opened vein)

	Flow ratios		
	$\frac{S.t. + S.n.}{S.t.}$	$\frac{S.t. + I.t.}{S.t.}$	$\frac{I.t. + I.n.}{I.t.}$
m =	1 96	2.06	1 97
s = ±	0 073	0 203	0 16[illegible]
e = ±	0 023	0 0[illegible]8	0 083
n =	10	9	4

m = arithmetic mean.
s = standard deviation.
e = standard error of the mean.
n = number of experiments.
S.t. = superior temporal vortex vein.
S.n. = superior nasal vortex vein.
I.t. = inferior temporal vortex vein.
I.n. = inferior nasal vortex vein.

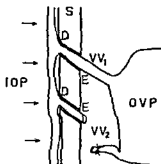


Fig 4 Schematic presentation of the features pertinent to the discussion of the outflow problem. *S* sclera, *OVP* orbital venous plexus, *VV* unopened vortex vein, *VV* opened vortex vein. At *D* the veins escape the full influence of the intraocular pressure *IOP* At *E* the veins leave the sclera.

General discussion

Direct determination of the blood flow through the uvea

The present findings indicate that at very low IOPs the flow from an opened vortex vein gives no measure for the uveal blood flow This is due to the fact that under such conditions the opened vessel drains more blood than the unopened veins The following consideration will show that the contribution of an opened vortex vein to uveal drainage is also influenced by the rate of blood flow through the uvea and the extraocular vortex venous pressure.

The pressure drop for blood flow through the uvea obviously equals the pressure in the arteries entering the uvea minus the pressure in the veins leaving it The latter pressure was not known However D in Fig 4 represents the place where a vein leaves the uvea At this point the vein probably also leaves the full influence of the IOP As long as there is flow through the vessel the venous pressure P_{VD} at this point cannot fall permanently below IOP as the vein is then compressed and the flow ceases As the vein is very thin walled it can be assumed for the sake of simplicity that it is kept open by a negligible distending pressure

Thus

(1) $$P_{VD} \geqq IOP$$

But P_{VD} also equals the sum of the extraocular vortex venous pressure P_{VE} at *E* in Fig 4 and the pressure drop from *D* to *E* This pressure drop equals the flow *I* through the vein multiplied by the vascular resistance *R* between *D* and *E*

Thus

(2) $$P_{VD} = P_{VE} + I \cdot R \geqq IOP$$

The extraocular vortex venous pressure in rabbits placed horizontally has been reported by Seidel (1937) to be 10—15 (—25) mm Hg as referred to the level at the eye (These figures are probably somewhat high as will be discussed but the pressure is definitely positive) The fact that the flow through

a vortex vein did not change when it was opened at IOPs of 10—15 mm Hg or more indicates that under such conditions a reduction in P_{VE} did not influence P_{VD}. This suggests that P_{VD} equalled IOP before the vein was opened as well as after. It seems reasonable then to assume that under certain conditions the resistance between D and E may be influenced by partial collapse of the vessel. To facilitate the discussion equation (2) may be rewritten as

$$(3) \qquad P_{VD} = P_{VE} + I \cdot (R_{m.n} + R_{oll}) \geqq IOP$$

where R_{oll} is the part of the resistance between D and E caused by collapse and $R_{m.n}$ is the resistance between D and E when the vein is fully expanded, which it is assumed here to be at a very small distending pressure.

When P_{VD} is higher than IOP the factor R_{oll} is zero. Opening of a vortex vein under such conditions reduces its P_{VD}, which gives an increase in flow from the arteries and shunting of blood from veins within territories normally drained by the unopened veins. When P_{VD} equals the IOP, R_{oll} is zero in the limit situation when $P_{VE} + I \cdot R_{m.n} = P_{VD} = IOP$, but is otherwise positive. Opening of a vein under such conditions obviously gives an increase in R_{oll}.

Now, if I or P_{VE} or both are increased above the levels in the present experiments at an unchanged IOP this may provoke a rise in P_{VD} above IOP also at IOPs of 10—15 mm Hg or more. This will always be the case in the limit situation referred to, but obviously may occur even if R_{oll} initially is positive but not large enough to compensate for the changes in question. Under such conditions the flow from an opened vein no longer gives a measure of the uveal blood flow, as pointed out.

Nothing was done in the present experiments to increase I or P_{VE} above the levels normally found in anaesthetized rabbits maintained at a blood pressure of 70—110 mm Hg. The present results therefore make it justifiable to determine the flow through the uvea as four times the flow from an opened vein only under certain restricted conditions. The IOP has to be 10—15 mm Hg or more and P_{VE} and uveal blood flow have to be normal or low.

It will be demonstrated elsewhere that at normal and high IOPs the procedure is adequate also at relatively high P_{VE}s and flow rates (BILL 1962 d).

In some additional experiments it was found possible to cannulate an opened vortex vein within its intrascleral path without changing the flow. In these experiments it was found that the flow through the cannulated vein ceased at an intrascleral venous pressure some 10—15 mm Hg above the IOP. This indicates a low resistance in the venous shunts within the uvea and emphasizes that the flow from an opened vortex vein gives a measure of the blood flow through the uvea only if P_{VD} in all the uveal veins equals IOP.

It should be pointed out that even if the pressure and flow requirements mentioned are fulfilled, considerable errors may be introduced in a single experiment. This is due to the fact that the flow through the vortex veins is not quite equal, as indicated in Fig. 3. However, in series of experiments such

errors will be of no importance as there are no systematic differences in flow between the veins

It could not be determined in the present experiments to what extent the surgical interference with the eye changed the reactivity of the uveal vessels There was no complete loss of vascular tone however as indicated by the finding in some additional experiments that acetylcholine given intraarterially increased the blood flow through an opened vortex vein

In many experiments it was found that in spite of stable arterial and intra ocular pressures the flow from an opened vortex vein tended to decrease shortly after the vessel had been opened The decrease which could not be prevented by larger doses of heparin was probably due to clotting as the flow could be restored by touching the opening with a glass rod Frequent mechanical freshenings of the opening as a rule made it possible to de termine the flow through the uvea for more than 30 min

The blood flow from a vortex vein usually decreased if the blood pressure fell at an unchanged IOP or if the IOP increased at a stable blood pressure It appears that the great variability in results obtained by earlier investigators determining the flow from an opened vortex vein in rabbits may have been caused by differences in intraocular and blood pressures and in the efficiency of the anticoagulants used Differences in anaesthesia and handling of the eye can be presumed to have given rise to the pressure differences and possibly also to differences in vascular tone

Comment on venous pressures

The regulation of the uveal venous pressure and the extraocular vortex venous pressure will be discussed elsewhere (BILL 1962 d) It should be pointed out however that the present findings indicate that normally and at high *IOPs* the venous pressure in the proximal part of the uveal veins is little different from the *IOP* This is in accordance with a rather general belief and in conflict with theories of SONDERMANN (1929 1932) KISS (1943) and VILSTRUP (1952)

The extraocular vortex venous pressure just outside the eye as determined with a pressure chamber technique was reported by SEIDEL (1937) to be 10—15 mm Hg under essentially the same conditions as in the present experi ments Values as high as 25 mm Hg were found if the eye was irritated as it was in several of the present experiments In the present study P_{VD} equalled *IOP* even at *IOPs* of 10—15 mm Hg As P_{VE} must be lower than P_{VD} it follows that P_{VE} was lower than in SEIDEL s experiments

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From the Institute of Physiology, University of Uppsala, Uppsala, Sweden

Studies of the Heated Thermocouple Principle for Determinations of Blood Flow in Tissues

By

Anders Bill

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Abstract

Bill, A. *Studies of the heated thermocouple principle for determinations of blood flow in tissues.* Acta physiol. scand. 1962. 55. 111—126. — A study was made in models of some of the factors influencing the adaptability of the heated thermocouple principle for blood flow determinations in tissues. The apparent thermal conductivity was determined with conventional technique. The tissue blood flow was simulated by flow of water in polyethylene tubes embedded in gelatin blocks and through holes in gelatin blocks. The findings indicate that there can be no standard type of relationship between the apparent thermal conductivity increment and the flow in any tissue into which the probe is blindly introduced. It is concluded from the results that the probe is most suitably placed when influenced only by flow in great numbers of capillaries and very small veins. For quantitative determinations the probe has to be standardized in each experiment. Standardizations will apply only under certain conditions, which are discussed.

During studies of the circulation of blood through the uvea it was considered that it would be of interest to find a procedure permitting determinations of relative changes in blood flow through the choroid and the anterior uvea separately. An attempt was made to utilize the heated thermocouple principle of Gibbs (1933), extensively used for determinations of the blood flow within tissues (for references see Graf and Rosell 1958). The results obtained in preliminary experiments made it seem of interest to determine by means of model experiments why the heated thermocouple was sensitive only in some cases to changes in blood flow. The present paper reports such a study.

When the heated thermocouple method is applied to measurements of the blood flow through tissues the probe used is introduced into a system in which the heat transport is extremely complicated. The probe will be influenced by

vessels of different size each vessel having a variable diameter In addition the flow may be orientated differently in the tissue In the choroid the conditions are somewhat more simple All the vessels are relatively small and there is a main direction for the blood flow in the larger vessels which is the same in the arteries and the veins It is also known that in the choroid the calibre of the vessels is not easily influenced (WUDKA and LEOPOLD 1956)

In part the models used in the present work were designed to resemble the conditions in the choroid The findings will nevertheless, contribute to an understanding of the circumstances under which heated thermocouples can be used for flow determinations in general

The heated thermocouple principle is based on the fact that the blood flow through an organ cools a heated body introduced into the tissue The greater the flow the more efficient is the cooling

Gibbs s procedure was to introduce into the tissue a constantan needle part of which was heated with a constant current The temperature difference Δt between the heated needle and unheated tissue was determined with a thermocouple Two procedures were used

1 The reference junction was thermostatically controlled outside the animal s body and the temperature of the constantan needle was determined repeatedly when heated t_H and unheated t_U The value of Δt in each situation was determined from the associated values of t_H and t_U The heating obviously had to be intermittent

2 Both the needle and the reference junction were introduced into the tissue, the reference junction being placed at such a distance from the needle that the heating did not affect it In the ideal case possible changes in tissue temperature were automatically compensated for as they affected both the junctions The heating could then be constant and Δt could be determined continuously

GIBBS emphasized that the method could only be used for determinations of qualitative changes in blood flow if the probe was not standardized in each experiment

The need for a standardization in each experiment was a very great disadvantage It therefore seemed a great improvement when GRAYSON (1952) reported that with a modification of GIBBS s procedure, it was possible to determine the apparent increase in thermal conductivity $\Delta\lambda$ which was caused by the blood flow and that $\Delta\lambda$ was proportional to the flow In GRAYSON s modification of GIBBS s method the heating current was modified to maintain a constant Δt of 1 C In a more convenient modification of HENSEL and RUEF (1954) Δt was recorded continuously at a constant heating current

At zero flow the true thermal conductivity λ_0 of the tissue can be determined

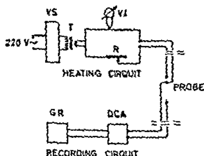


Fig 1 Schematic presentation of the set up VS voltage stabiliser T transformer VJ vacuo junction with galvanometer R rheostat In the probe stippled lines represent constantan wire unstippled lines copper wire GR graphic recorder DCA, direct current amplifier

where a is an instrumental factor which can be determined in a substance with known thermal conductivity and I is the heating current

When there was flow through the tissue the heating current had to be increased in GRAYSON's experiments to maintain Δt at 1 C In Hensel's modification Δt decreased Thus there was an apparent increase in conductivity above k_0 the increase being ΔK (In fact the conductivity obviously could not be determined as there was convectional heat transport within the system)

GRAYSON found that the heat transport from the heated body was influenced in a detectable way only by a tissue collar with a radius of 1 5—3 mm.

Experiments verifying or contradicting GRAYSON's findings concerning the relationship between the blood flow and Δk have been repeatedly reported in the literature (See discussion)

Methods

In the present experiments absolute values for conductivity data as a rule were not calculated In each experiment the heating current was kept constant The inverse value of Δt then gave a measure of the conductivity in arbitrary units

The apparatus used to deliver the heating current and the probes used did not essentially differ from those used by GRAYSON (1952) and HENSEL and RUEF (1954) see Fig 1

The AC mains voltage was stabilized and transformed to 6 volts The current to the probe could be varied with a rheostat and determined by means of a vacuo junction

The probe used consisted of a 12 mm long constantan wire, 0 10 mm in diameter which was soldered at each end to copper wires 0 12 mm in diameter and also to another copper wire of the same diameter crossing over the constantan wire 2 mm from one end The 2 mm part of the constantan wire was heated and the EMFs caused by the temperature difference between the ends of the 10 mm part of the constantan wire were amplified in a microvoltmeter (Pye 11340 or Hewlett Packard 425A) and recorded with a graphic recorder (Varian G 10) The copper wires were enamelled and a varnish was used to provide electrical insulation for the probes

Experiments

In the main group of experiments the blood flow through a vessel was simulated by a flow of water from a reservoir through a polyethylene tube The probe and the tube were embedded at least 10 mm below the surface in blocks of 20 per cent gelatin simulating tissue All experiments were performed with

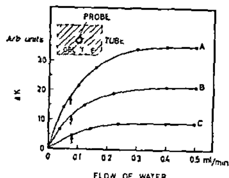


Fig 2 The relationship between ΔK and flow of water The model used is shown inset in the figure The probe was placed close to and in parallel with the polyethylene tube which had an inner diameter of 0 36 mm wall thickness 0 20 mm Curve A was obtained when there was no extra layer of polyethylene added to the tube curve B when one extra layer thickness 0 20 mm had been added to the tube wall and C when two extra layers had been added Arrows at value F_{50}

the whole system at room temperature except the part electrically heated The heating current was such as to give a Δt of about 3 C at zero water flow The heated junction was placed downstream The flow rates investigated were not high enough to produce turbulence in any of the tubes used It was determined

1 what was the relationship between ΔK and the flow through a polyethylene tube when the probe was placed outside the tube

2 how this relationship was influenced by the thickness of the wall of the tube

3 how the relationship was influenced by the inner diameter of the tube

4 how distant vessels influenced the probe if there was or was not flow through vessels nearer the probe

The relationship between ΔK and the flow, and the influence of the thickness of the wall on this relationship

Procedure The probe was placed as close as possible to a tube with an inner diameter of 0 36 mm wall thickness 0 20 mm, and the relationship between ΔK and the flow from the tube was determined

One extra layer of polyethylene, 0 20 mm thick, was then added to the wall o the tube and the relationship between ΔK and the flow was determined again with the same probe and heat production The procedure was also repeated with two extra layers of polyethylene each extra layer being 0 20 mm thick

Results Fig 2 shows that when the isolating layer was incrased the general sensitivity of the probe decreased Each of the curves A B and C reaches an asymtote at high flow rates which makes it impossible to determine a flow value for the tube at which a further increase in flow no longer changes ΔK For each curve it is possible however to determine approximately the flow value at which 50 per cent of the maximum change in ΔK is reached This value F_{50} can be seen to be essentially the same in all the 3 experiments

In the following it will be shown that F_{50} is dependent essentially on the inner diameter of the tube provided the same fluid and tube material is used. F_{50} then can be used to define a flow range for each tube within which moderate

changes in flow give reasonable changes in ΔK in relation to the maximum ΔK which can be produced by *flow in the tube* — that is an arbitrary acceptable flow range This range will be defined as from zero to twice F_{50} in the following description It is highly arbitrary as in fact not relative changes in ΔK but absolute changes *will determine the true acceptable flow range* However the absolute change in ΔK produced by a certain change in flow in each particular tube is influenced also by the distance between the tube and the probe as demonstrated above and *in addition by the diameter of the tube (vide infra)* In models it might then be possible to define a truly acceptable flow range for each tube taking into account the distance between the tube and the probe and also the characteristics of the probe and *the heating produced Such an elaborate* definition would be of no practical use however as in a tissue the truly acceptable flow rate for each particular vessel is influenced by still more factors which vary from one experiment to another *One such factor of great importance* is that in a tissue, there will be differences in temperature between different places and the temperature at each place will change somewhat e g with changes in *the blood flow Therefore when the constant heating procedure is* used there will always be a slightly varying temperature difference between the two junctions which is not caused by the heating of the probe When the intermittent heating procedure is used *there will be slight changes in the local* tissue temperature which are not caused by the heating Accurate flow determinations will be possible only if the error in the determinations of Δt due *to these factors is relatively small The larger the errors the larger must be* the changes in Δt which are caused by the cooling effect of the blood flow

The main merit of the arbitrary acceptable flow range concept is that it will *facilitate the following discussion of which vessels that may permit flow determi*nations with heated thermocouples and what type of relationship between ΔK and flow that can be expected under different conditions

The relationship between F_{50} and the inner diameter of the tube

These experiments will show that only very small vessels permit determinations of moderate changes in the blood flow with a heated thermocouple The relationship between ΔK and the flow was determined in different tubes with inner diameters ranging from 0 12 mm to 1 50 mm

Procedure The probe was embedded in the gelatin as close to the tube as possible and the relationship between ΔK and flow was determined In each experiment the value for F_{50} was determined To permit comparisons with calculated data for mean linear velocities of streaming blood in vessels, the mean linear velocity V_{50} of the streaming water corresponding to F_{50} was determined from the flow and the cross sectional area of the tube

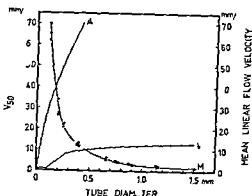


Fig 3 The relationship between V_{50} and the inner diameter of the tube in the model experiments Solid circles represent values obtained with flow of water through polyethylene tubes embedded in 20 % gelatin Curve M connects these values Solid squares represent values obtained with flow of water through tubes in 10 or 30 gelatin open circles values obtained with flow of blood in polyethylene tubes embedded in 20° gelatin, open squares values obtained with flow of water through channels in 20 gelatin Approximate values for the mean linear flow velocities in tissue vessels are plotted against the corresponding diameters Curve A gives the values for the arterial side curve V those for the venous side

Results The same type of relationship between ΔK and the flow was found in all experiments as in the preceding series V_{50} was inversely related to the tube diameter as shown in Fig 3

As could be expected the change in local conductivity which could be produced by flow in narrow tubes was smaller than that in wider ones It was therefore possible to determine Γ_{50} for wider tubes with greater precision than that for very narrow tubes as can be seen in Fig 3

Human blood was used instead of water in some experiments The results obtained were essentially the same as with water flow in the same tube as shown in the figure In other experiments the probe was embedded in 10 or 30 per cent gelatin and in yet other experiments the water was made to flow through holes in gelatin blocks The results obtained also in these experiments were very little different from those expected from the relationship found in the main series of experiments which is also clear from the figure

The present findings show that the wider the tube or the vessel that influences the probe the lower is the upper limit of the linear velocity range corresponding to the arbitrary acceptable flow range

As the total cross-section of the circulatory system decreases and the diameter of the individual vessels increases as the heart is approached the mean linear velocity in general will be larger the wider the vessel both on the arterial and the venous side Thus for both arteries and veins there will be an upper limit for the size that can be expected to permit flow determinations with heated thermocouples It is possible to determine approximately from known data what the mean velocity may be in the vessels within the tissues In Fig 3 the velocities in the vessels on the arterial side and those on the venous side are each represented with one line (Data from Green 1950) From the intercepts between the vessel lines and the tube line it is possible to determine which vessels normally have a flow velocity that equals V_{50} in the corresponding poly

ethylene tube If the probe is placed in such a way as to be influenced only by the flow through such a vessel and the diameter of the vessel is assumed not to change its diameter with increased flow, it is clear from the first type of experiment that the flow through the vessel may be increased to twice the normal value before the arbitrary acceptable flow range is exceeded

Obviously the flow in vessels smaller than those discussed above may increase even more above the normal level before the arbitrary acceptable flow range is exceeded while vessels which are moderately larger than those discussed first easily will show a flow increase which brings the flow above this range Arteries with a diameter of about 0 23 mm and veins with a diameter of about 0 65 mm normally have a flow that is just at the upper limit of the arbitrary acceptable flow range for these calibres

However, as the absolute change in local Δk which can be produced by the flow through a tube is related to the inner diameter the truly acceptable flow range in very small vessels is a smaller part of the arbitrary acceptable flow range than in larger ones Thus an individual capillary which has a very high arbitrary acceptable flow range can be presumed to have no truly acceptable flow range at all due to the small Δk which is produced even by very high flow rates through such a small vessel Intermediate sized and larger tissue vessels however may have a truly acceptable flow range of the same order as the arbitrary acceptable flow range This could be concluded from a comparison between the absolute values for Δk calculated in some of the present experiments and the values for ΔK found in tissues by earlier investigators

The values for flow velocity in different parts of the vascular system are rough approximations as pointed out by GREEN and refer to the situation in dogs There seems to be no reason however why the conditions in humans and laboratory animals like cats and rabbits should be essentially different from those in dogs

Influence of near and distant tubes

The purpose of these experiments was to demonstrate that the influence on the probe of the flow through a certain vessel was dependent on whether there was flow also in other vessels close to the probe

Procedure The probe was placed as shown inset in Fig 4 so that it was quite close to two of a row of 4 polyethylene tubes (inner diameter 0 36 mm) implanted in 20 per cent gelatin

The Δk produced by a relatively high flow in each tube separately was determined with a flow of 0 4 ml per minute through each tube The same flow was then produced through all the tubes in different combinations as indicated in Fig 4 and Δk produced under such conditions was determined

Results As could be expected the flow through the tubes nearest the probe influenced the probe to a much larger extent than that through the more distant tubes When there was flow only in the most distant tube it influenced the

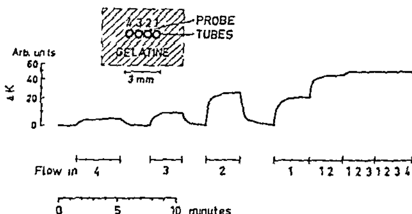


Fig 4 The influence of flow through distant tubes on the probe when there is not respectively is, flow through tubes placed in between the distant tubes and the probe The model used is shown inset in the figure The probe was sited close to one of 4 parallel tubes placed deep in gelatin Inner diameter of the tubes 0 36 mm wall thickness 0 20 mm The flow rate in each tube was zero or 0 4 ml per minute

probe considerably but when there was flow also through the nearer tubes its influence was hardly detectable Further, even the flow through the tube 3 influenced the probe to a far less extent in this situation Similar experiments with other tube diameters gave the same results It can be concluded that the probe when placed in a tissue will be influenced essentially by the very nearest vessels More distant screened vessels of the same size or smaller will influence the probe very little even if the distance to the probe is only some 1 5—2 mm

The relationship between ΔK and the flow in a two-tube system

These experiments were performed to demonstrate that if the probe is influenced by the flow through more than one tube the findings in the single tube experiments concerning F_{60} still apply

Procedure The probe was placed close to two tubes of the same inner diameter (0 36 mm) embedded in gelatin Two types of tube arrangement were used see models inset in Fig 5 and 6 The relationship between ΔK and flow was determined first for each tube separately and then with the same flow rates in both the tubes at the same time

Results Fig 5 shows the result of an experiment with the probe placed between the tubes The ΔK produced by a certain flow rate in each tube when there was flow in both the tubes was not essentially different from the sum of the ΔKs produced by the same flow rate in each of the tubes separately There was also no essential difference in F_{60} for each tube when there was flow through both the tubes as compared with when there was flow through only one tube

Fig 6 shows the result of an experiment in which the probe was placed close to one tube the other tube being sited on the other side of the first mentioned tube

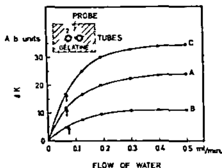


Fig 5 The relationship between ΔK and the flow when the probe was influenced by the flow in two tubes The model used is shown inset in the figure The probe was placed close to one of two polyethylene tubes inner diameter 0 36 mm wall thickness 0 20 mm the other being sited at some distance from the probe but not screened by the closest tube Curve A was obtained with flow only through tube 1 curve B with flow only through tube 2 and curve C with flow through the two tubes at the same time. The abscissa gives the flow through each tube When there was flow only through one tube the other was filled with water Arrows at value F_{50}

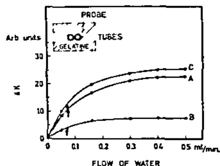


Fig 6 The relationship between the ΔK and the flow when the probe was influenced by flow through two tubes. The model used is shown *inset in the figure The probe* was placed close to one of two polyethylene tubes inner diameters 0 36 mm wall thickness 0 20 mm placed in parallel Curve A was obtained when there was flow only through tube 1 curve B when there was flow only through tube 2 (The tube without flow was filled with water) Curve C was obtained when there was the same flow through both the tubes The abscissa gives the flow through each tube Arrows at value F_{50}

The ΔI produced by the simultaneous flow in both the tubes was considerably less than the sum of the ΔKs found with flow through each tube separately F_{50} however was not changed in a detectable way

Thus if a probe in a tissue is influenced by two vessels of the same size the arbitrary acceptable flow range for each vessel is the same as it would have been had there been only one vessel provided the flow changes are the same in the two tubes

These experiments indicate that in tissues the probe will be placed as favourably as possible when influenced by the flow in a great many very small vessels preferably capillaries venules and very small veins Each of the vessels then will have a high arbitrary acceptable flow range and the absolute changes in ΔK which are produced by moderate changes in flow through the tissue will be large due to the large number of vessels

The time taken to reach the steady state ΔI

The time taken for ΔI to reach a new steady level after a large change in flow was several minutes It was found however that 90 per cent of the total change that was produced in ΔK was wrought within about one minute irrespective of which model was used and irrespective of the tube diameter and the distance between the probe and the tube

Table I Thermal conductivities of tissues and substances used in the model experiments

Substance	Temp C	Thermal conductivity $\times 10^{-4}$ cal cm^{-1} sec^{-1} C^{-1}	Source
10% gelatin	37	12 2	GRAYSON (1952)
20 gelatin	37	11 5	GRAYSON (1952)
30% gelatin	37	11 0	GRAYSON (1952)
Polyethylene	—	8 0–11 0	FRITZ (1957)
Water	20	14 2	HODGEMAN (1958)
Liver rat	37	11 8	GRAYSON (1952)
Liver dog	37	11 9–12 0	GRAF et al (1957)
Kidney rabbit	37	12 0	GRAYSON (1952)
Muscle, human	about 32	13 7	HENSEL et al (1954)
Blood human	36 8 – 39 4	12 1	SPELLS (1960)
Steel	18	115 0	HODGEMAN (1958)
Ethyl alcohol	20	4 0	HODGEMAN (1958)

General discussion

Comment on substances used in the present study

The majority of experiments in the present study were performed with substances which have thermal conductivities of the same order as that found in the blood and most tissues see Table I

It then seems quite justifiable to conclude from a consideration of the thermal conductivity data in Table I and the results demonstrated in Fig 3, that the findings in the polyethylene tube experiments have relevance also from a quantitative aspect in living tissues This has in fact already been assumed in the preliminary discussion of each experiment It should be pointed out however that the results have no general application quantitatively as indicated by the findings in some additional experiments In some of these the polyethylene tube was replaced with a steel canula in others ethyl alcohol was used instead of water The values found for V_{50} were some 50—80 per cent higher than those in the main group of experiments

Remarks on flow determinations without standardizations

There has been considerable disagreement among earlier investigators concerning the relationship between ΔK and blood flow It was stated repeatedly by GRAYSON and co workers that in different tissues such as liver brain and kidney, ΔK is approximately proportional to the flow at all physiological flow rates For references see DOSEKUN GRAYSON and MENDEL (1960) The same type of relationship was found in perfused spleens by HENSEL and RUEF (1954) This type of relationship it was pointed out made it possible to determine semi

quantitative that is percentage changes from some normal value in local blood flow HENSEL and BENDER (1956) reported that applied to the skin, the method permitted even quantitative determinations of the blood flow in ml/100 ml skin/min Such information was obtained simply by multiplying the values for ΔK by a constant factor

LINZELL (1953) on the other hand reported that in perfused kidneys ΔK was proportional to the flow only at low flow rates At higher flow rates ΔK still increased with the flow but less than required for proportionality In model experiments he showed that if the probe was introduced into the streaming fluid in glass tubes ΔK was proportional to the flow at low flow rates and to the square root of the flow at intermediate flow rates At still higher flow rates ΔK did not change in a detectable way for moderate changes in flow It was also found that it was not the flow but the mean linear flow velocity of the streaming fluid which determined ΔK Thus irrespective of the tube diameter the line representing the relationship between ΔK and the mean linear flow velocity *reached an asymtote at about 9 cm/second*

The same type of relationship between ΔK and the flow as found in LINZELL's kidney experiments was found in the liver by GRAF GOLENHOFEN and HENSEL (1957) and in perfused human bone marrow by GRAF and STEIN (1958) In muscles however GRAF and ROSELL (1958) again found ΔK to be approximately proportional to the flow

MOWBRAY (1959) reported that it was possible to calibrate heated thermocouples in models with flow through glass wool and to use such calibrations to obtain measures of tissue blood flow in ml/100 ml/min both in the thyroid and in muscles NIESEL and KONSTAS (1959) finally assumed that in the rabbit uvea Δt decreases linearly with increasing flow

The present model experiments show that if a probe introduced into a tissue is placed close to some relatively large vessels the apparent thermal conductivity increase caused by the flow through these vessels will be much larger than if the probe is sited so as to be influenced only by flow in a few and very small vessels in the same tissue Thus the findings of MOWBRAY (1959) must have been obtained in some experiments in which the probe was placed in such a way in muscle and thyroid that by chance the standardization performed in the models applied A general application of such standardizations is excluded from the present work and also from the work of GRAF and ROSELL (1958) These investigators demonstrated that in muscle tissue it is possible to place a probe in such a way that the normal flow through the tissue gives very different values for ΔK in the same muscle

It seems probable also that the finding of HENSEL and BENDER (1956) that the blood flow through the skin can be determined by multiplication of the apparent thermal conductivity increase with a constant factor has no general applicability in determinations of skin blood flow The vessels in the skin which are likely to dominate the heat transport from the probe are those in the papillary

layer situated under the epidermis That these vessels will be of the greatest importance was indicated by the findings in the multitube model that flow through the nearest vessels reduced the influence of distant screened vessels

The thickness of the epidermis isolating the probe from the vessels in the papillary layer is quite variable from one individual to another and from place to place in the same individual As a rule the thickness varies between 0 03 mm and 0 20 mm but it can reach values above 1 mm The experiments performed in the present study with different wall thicknesses show that such differences in thickness of the epidermis can be expected to give large differences in ΔK It seems very likely that the number of vessels in the papillary layer varies from one place to another which also will tend to give errors

For practical reasons HENSEL and BENDER chose to calibrate the probe when *attached to the skin of the third finger It seems probable that the reason for the* good agreement in ΔK/blood flow relationships between different individuals was similarity in age and occupation, of the persons investigated which may have given a similar epidermal thickness and also a similar number of vessels in the papillary layers of the fingers examined

It seems justifiable to state that in general, determinations of absolute values for blood flow through tissues cannot be made with the heated thermocouple principle unless the probes are standardized in each individual experiment

The many reports about proportionality between ΔK and the local blood flow also seem hard to understand in the light of the present findings These unequivocally show that there can be no such standard type of relationship between ΔK and the local blood flow in any tissue into which the probe is introduced blindly In some positions the probe may be influenced by only very small vessels and, in such a case any change in flow through the tissue will change the local ΔK In other positions the probe may be influenced by a larger tissue artery or vein Under such conditions local ΔK will change very little for moderate changes in flow as the normal flow in such vessels is much above F_{50}

What is then the explanation for the many reports of a linear relationship between ΔK and flow? Several factors may contribute to give this type of relationship quite often in tissues *Firstly it is obvious from the present experiments* that if the probe is placed so as to be influenced only by the flow in very small vessels the curvature of the line representing the relationship will be very *small as the maximum physiological flow velocity in all the vessels is far below* V_{50}

Secondly the total number of vessels open at high flow rates may be higher than that at lower flow rates This obviously *will tend to give an* extra increase in ΔK, as the flow through the tissue is increased.

Thirdly a distension of the vessels will tend to give the same effect as illustrated in Fig 7 As the flow increases there is a *shift from one ΔK/flow curve* to another simulating a long linear rise

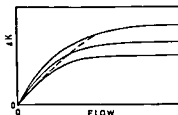


Fig 7 The effect of distension of the vessel on the relationship between ΔK and flow Unbroken lines, hypothetical curves showing the relationship between ΔK and flow in a vessel at three different states of distension Broken line the same relationship when the vessel is permitted to dilate in a continuous fashion

It is of interest to note here the proportionality between ΔK and the blood flow reported by HENSEL and BENDER (1956) to apply in skin and by GRAF and ROSELL (1958) in muscles Placed on the skin of the finger, as in HENSEL's experiments the probe can be expected to be influenced essentially by the small vessels in the superficial layer in the corium as mentioned The majority of these vessels are very small and the number of vessels influencing the probe will be large The present findings indicate that this is ideal in flow determinations with heated thermocouples and may often give proportionality between ΔK and flow

The results of GRAF and ROSELL (1958) may appear to differ somewhat more from what can be expected It was stated by these authors that in muscles there is approximative proportionality between ΔK and the blood flow if the probe is placed so as to measure a ΔK above a certain value at normal flow In dissections of the tissue it was found that under such conditions the probe was placed close to a macroscopically visible vessel When placed so that the ΔK produced by the normal flow was below the limit value it was found to be sited in capillary areas without larger vessels In the latter position the changes in ΔK which were produced by changes in flow were so small as to make errors in the determinations of Δt play a great role and the position was regarded as not acceptable The present findings show that the ΔK produced by the normal flow gives very little information about whether a position is good or bad If the probe is placed e g close to an artery or a relatively large vein the ΔK produced by the normal flow in these vessels will be great but moderate changes in flow may give very little change in ΔK because of the size of the vessels and the high mean linear flow velocity at normal flow rates in these vessels

However the experiments of GRAF and ROSELL were performed in cats hind muscles and the blood and the tissue were not thermostatically maintained at a constant temperature. It seems very probable that in such a relatively small structure as that investigated only places with similarities in vascular arrangement may be isothermal under such conditions It can further be presumed that for anatomical reasons the chance of placing both the junctions close to larger veins or arteries will be smaller the smaller is the organ

The reason for the proportionality reported may then be that places where the heated part of the probe was influenced by relatively large vessels were not

investigated due to the necessity of finding isothermal places for the two junctions and the small probability of finding such places near large vessels The authors report in fact that the position of the probe had to be changed 10—20 times before a good place for it was found

It can be concluded that when the probe is introduced blindly into a tissue one cannot expect a standard type of relationship between $\Delta\lambda$ and the blood flow in any tissue

Flow determinations with standardizations

The preceding discussion shows that, for studies of semiquantitative changes in flow it is necessary, as a rule, to standardize the probe in each experiment This can be done only if there is a procedure by which the flow can be changed in a known way

For quantitative determinations of the blood flow through a tissue with heated thermocouples these have to be calibrated with some method for quantitative flow determinations

In either type of standardization there is no need for calculations of data for apparent thermal conductivity as it is quite satisfactory to know the relationship between Δt and the flow

However the present findings indicate that standardizations of the probes will be reliable only under certain conditions the spatial relationship between the probe and the streaming fluid in the tissue must be the same at each particular flow irrespective of which factors influence the probe This requirement is not fulfilled e g if the probe is placed close to an arteriole varying greatly in diameter with changes of sympathetic tone but little with changes in pressure head for flow through the tissue

A calibration of the probe in such a case performed e g by varying the tissue pressure will not apply for changes in blood flow produced by stimulation of the sympathetics as the same flow through the tissue in the two cases affects the probe in very different ways During the calibration it is influenced by a relatively wide vessel with a moderate mean linear flow velocity of blood In the latter case the vessel is narrow and the mean linear flow velocity of the blood may be several times higher

As the vessels dominating the heat transport from the probe are those closest to the probe it must be suspected that when the probe is used for measurements of blood flow within tissues with flow also of other fluids convectional heat transport with these fluids may now and then introduce errors This may be the case e g when in liver experiments the probe is placed close to a bile duct In such unfortunate experiments standardizations of the probes will have no meaning

If the tissue within which the flow is measured has a tendency to change its extravascular volume with changes in blood flow then great errors may be introduced into the flow determinations A reduction in flow under such condi

tions may very well even increase $\Delta\lambda$ if it causes a reduction in the tissue isolating the probe from the vessels

The present experiments largely confirm GIBBS's somewhat pessimistic statement concerning the usefulness of the heated thermocouple principle If a standardization is not performed in each experiment the method as a rule can be used essentially for determinations of qualitative changes in blood flow They also demonstrate

1 that the standardizations may not always apply

2 that the probe may be placed in such a way that it is insensitive to moderate changes in flow even if it is close to vessels

3 that qualitative errors may be introduced even by moderate changes in extravascular volume and

4 that under unsuitable conditions the convectional heat transport from the probe may be dominated by flow of fluids other than blood

Summary

The adaptability of the heated thermocouple principle to quantitative or semiquantitative determinations of tissue blood flow has been studied in model experiments In all the experiments the probe was placed close to vessels in gelatin blocks

1 When the probe was placed close to a polyethylene tube embedded in the gelatin convectional heat transport associated with the flow caused an apparent increase $\Delta\lambda$ in thermal conductivity There was a detectable change in $\Delta\lambda$ for a moderate change in flow only within a small range of low flow rates At higher flow rates the line representing the $\Delta\lambda$/flow relationship reached an asymtote Extra layers of polyethylene added to the wall of the tube reduced the general sensitivity but the flow rate F_{50} at which 50 per cent of the maximum change in $\Delta\lambda$ was reached was not essentially changed

2 In similar experiments with tubes of different diameters or with flow through holes in gelatin blocks it was found that the mean linear velocity corresponding to F_{50} for each tube was inversely related to the diameter of the tube or the hole In the tissue vessels the mean linear flow velocity increases as the heart is approached Therefore there is for both arteries and veins an upper size limit for the vessels which will be acceptable in flow determinations Placed close to arteries about 0 18 mm in diameter or veins about 0 45 mm in diameter the probe is sensitive within a flow range from zero to about twice the normal flow

3 When the probe is influenced by the flow in two tubes placed one on each side of it the flow through each tube influences the probe to about the same extent as in single tube experiments If the two tubes are placed on the same side of the probe one screening the other the influence on the probe of flow through the screened tube is much reduced by flow through the nearer one

4 When the probe is placed so as to be influenced by flow in both distant and nearby tubes of the same size the flow in distant tubes screened by the nearby tubes is negligible even if the distant tubes are sited only about 1 5 mm from the probe

It is concluded that there is no standard type of relationship between $\Delta\lambda$ and the flow in any tissue into which the probe is blindly introduced A standardization thus has to be made in each experiment if more than qualitative information is sought.

It is pointed out that standardizations may not be valid under all conditions and that changes in the perivascular tissue isolating a probe from a vessel may give large and even qualitative errors

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From the Laboratory of Clinical Neurophysiology Department of Neurology
University of Lund Sweden

Tissue pO_2 and pCO_2 of the Cerebral Cortex, Related to Blood Gas Tensions

By

U Gleichmann[1] D H Ingvar D W Lubbers
B K Siesjo and G Thews[3]

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Abstract

Gleichmann U D H Ingvar D W Lubbers B K Siesjo and G Thews. *Tissue pO_2 and pCO_2 of the cerebral cortex releated to blood gas tensions* Acta physiol scand 1962 *55* 127—138 — Quantitative measurements of the pO_2 and the pCO_2 on the intact surface of the cerebral cortex were made in anesthetized dogs Simultaneous measurements of the cortical metabolic rate of oxygen and carbon dioxide as well as of the arterial and cortical venous pO_2 and pCO_2 (Gleichmann *et al* 1962) allowed calculation of the average tissue pO_2 and pCO_2 according to Thews (1960 a and b) The results indicate that the pO_2 and the pCO_2 electrodes measure tensions which are close to the calculated average tissue pO_2 and pCO_2 respectively

It has long been recognized that direct measurements of the tissue oxygen and carbon dioxide tensions could yield useful information on important events in the central nervous system A step forward was the development of polarographic electrodes for measurement of the oxygen tension in tissue (Davies and Brink 1942) Since that time a number of qualitative studies of the oxygen tension of the cerebral cortex have been performed (Davis McCulloch and Roseman 1944 Davies and Remond 1947 Mochizucki 1951 Meyer and Denny Brown 1955 *et seq* Ingvar Lubbers and Siesjo 1960 1962 Meyer and Gotoh 1961) Although quantitative measurements have been possible with the recessed type of platinum cathode (Davies and Brink 1942 Davies and Bronk 1957 *cf* Cater 1960) such measurements have not been performed systematically to any larger extent (See discussion)

[1] On leave of absence from the Institute of Physiology Cologne
Present adress Department of Applied Physiology Marburg
[3] On leave of absence from the Institute of Physiology K I

Recently a membrane covered recessed type of platinum cathode has been developed for intermittent quantitative measurement of the oxygen tension on the surface of a tissue such as the cerebral cortex (LUBBERS 1960) Also a method have become available for continuous, quantitative measurement of the carbon dioxide tension on tissue surfaces (HERTZ and SIESJÖ 1959, SIESJÖ 1961) Since these electrodes, when applied to the cerebral cortex measure the respective tensions at the pia arachnoidal surface no immediate conclusions can be drawn from such measurements as to the tensions in the tissue proper Such conclusions evidently awaits the experimental correlation between the tensions measured at the surface and those existing in the tissue proper

The distribution of a gas in a tissue can be analyzed theoretically with the help of a suitable diffusion model *e g* the tissue cylinder (KROGH 1919, OPITZ and SCHNEIDER 1950 THEWS 1953, 1960 KETY 1957, THEWS 1960 a) A practical advantage of such an analysis is the introduction of an average tissue tension which can be calculated from the tissue cylinder model This calculation requires knowledge of (1) the mean capillary diameter as well as the mean intercapillary distance of the particular tissue (2) the diffusion coefficient for the gas in the tissue (3) the tissue consumption or production of the gas ($rCMR_{O}$ and $rCMR_{CO}$) and finally, (4) the gas tensions along the capillary The consumption or production of a gas in the tissue can be calculated if the total content of the gas in arterial and representative venous blood as well as the tissue blood flow is known

Presently the above method to calculate the average tissue tension can be used for oxygen and carbon dioxide in the cerebral cortex of experimental animals *This has been made possible by the determination* of the diffusion coefficients for oxygen (THEWS 1960 b) and carbon dioxide (SIESJÖ and THEWS 1962) in cerebral gray matter as well as by the development of a repeatable method for the determination of regional cerebral blood flow (LASSEN and INGVAR 1961 1962) the capillary constants being known (HORSTMANN 1960) and methods for the determination of gas contents and tensions (SEVERINGHAUS and BRADLEY 1958 GLEICHMANN and LÜBBERS 1960) already at hand

The present work aimed at an interpretation of the tissue tensions measured on the surface of the cerebral cortex with the pO_2 and the pCO_2 electrodes by simultaneous measurements of the factors necessary for calculation of the average tissue pO and pCO$_2$ It will be shown that the tensions measured and those calculated agreed fairly well The conclusion is reached that the pO_2 and pCO_2 measured with electrodes of the present type and size come close to the actual average gas tensions in the tissue

Methods

1 Calculation of average tissue pO_2 and pCO_2

The calculation of the average tissue pO_2 and the average tissue pCO_2 is based upon the following diffusion model The tissue can be functionally divided into cylindrical areas which are supplied with centrally situated capillaries (KROGH 1919) The average

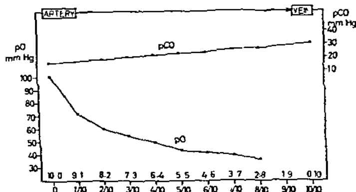


Fig. 1. Gradients of pO_2 and pCO_2 in a capillary. Ordinate: pO_2 and pCO_2 in mm Hg. *Abscissa*: Relative length of capillary with arterial end at 0/10 and venous end at 10/10. Measurements of pO_2 and pCO_2 were carried out after mixing arterial and venous blood in proportions given on the abscissa, *e. g.* 6/10 mean that 6 parts of arterial blood were mixed with 4 parts of venous blood.

capillary radius (R_1) for the dog is given as $2.5 \cdot 10^{-4}$ cm, the radius of the tissue cylinder (R_2) as $30 \cdot 10^{-4}$ cm (HORSTMANN 1960, *cf.* THEWS 1960 b). The consumption of oxygen and the production of carbon dioxide is assumed to have a temporal and spatial uniformity within the tissue. For such a steady state condition the differential equation for the diffusion can be solved. Following integration over the cross section of the tissue cylinder and along the capillary, the following formula is derived for the average tissue gas tension (as a function of the constants which determine the diffusion):

$$\bar{p} = \bar{p}c \pm \frac{A}{\lambda}\left[\frac{1}{8}(3R_2^2 - R_1^2) - \frac{R_2^4}{2(R_2^2 - R_1^2)}\ln\frac{R_2}{R_1}\right] \tag{1}$$

$\bar{p}$ = the average tension of the gas in the tissue, integrated across the cross section of the cylinder and along the capillary, in atm

$\bar{p}c$ = the average tension of the gas in the capillary, integrated through the length of the capillary, in atm

A = the consumption (with positive prefix) or the carbon dioxide production (with negative prefix) in ml/ml tissue/min

λ = the diffusion coefficient of KROGH in ml gas/cm/min/atm.

The term within the large brackets only depends upon the magnitude of R_1 and R_2, the values of which are given above. The equation can thus be simplified into

$$\bar{p} = \bar{p}c \pm \frac{A}{\lambda} 7.9 \cdot 10^{-6} = \bar{p}c + \Delta p \tag{2}$$

Δp is thus the difference between the average tissue tension and the average capillary tension. It varies only with the metabolic rate of the gas, provided the radius of the capillary is regarded a constant. In the present investigation the metabolic rate (A) was determined in each experiment through measurement of the regional cortical blood flow as well as of the arterio-venous differences of oxygen and carbon dioxide. The diffusion coefficients (λ) used were the following: $\lambda O_2 = 2.3 \cdot 10^{-5}$ ml/cm/min/atm (37° C) (THEWS 1960 b), $\lambda CO_2 = 5.4 \cdot 10^{-4}$ ml/cm/min/atm (37° C) (SIESJÖ and THEWS 1962).

The average tension of the gas in the capillary ($\bar{p}c$) can be obtained by graphical integration if the change of tension along the capillary is known. For oxygen this

change is dependent upon the part of the oxygen dissociation curve occupied The tension curve along the capillary can be determined in two ways the first of which was developed for the present purpose (LUBBERS and GLEICHMANN 1961)

The first procedure is based upon the assumption that the oxygen consumption (resp the carbon dioxide production) is identical in all tissue loci along the capillary Hence the decrease in oxygen concentration (resp the increase in carbon dioxide consentration) along the capillary should be uniform The decrease of the oxygen concentration and increase of the carbon dioxide concentration was thus mimicked by mixing arterial and venous blood in different proportions (10 to 0 9 to 1 8 to 2 etc) and the pO_2 and the pCO_2 of the mixtures were determined (Fig 1) Using the original venous blood at the tissue temperature no corrections were necessary The resulting curve clearly demonstrated the linearity of the physiological carbon dioxide dissociation curve

According to the second procedure the arterial and venous oxygen tensions were transformed into standard conditions and the corresponding values for oxygen content determined from the oxygen dissociation curve for the dog s blood (BARTELS and HARMS 1959) The difference in oxygen content between arterial and venous blood was then *divided into ten equal parts and the corresponding oxygen tensions* read from the dissociation curve were transformed into a curve illustrating the slope of tension in the capillary From the tension curves obtained with either method the mean capillary tension was obtained by graphical intergration

B Techniques and experimental procedures

The present experiments were performed on dogs after preliminary tests on dogs cats and rabbits. The experimental procedures have been reviewed in detail elsewhere (GLEICHMANN *et al* 1962) Only a few relevant facts will be recalled here. The regional cortical blood flow (rCBF) was measured according to LASSEN and INGVAR (1961 1962) the oxygen and carbon dioxide tensions in arterial and cortical venous blood according to GLEICHMANN and LUBBERS (1960 *cf* SEVERINGHAUS and BRADLEY 1958) and the total blood oxygen and carbon dioxide content by manometric determinations according to V SLYKE and NEILL (1924) Arterial and cortical venous blood samples were taken in paraffined and heparinized 2 ml all glass syringes from polyethylene cannulae mounted in the femoral artery and in the superior sagittal sinus respectively It was found necessary to remove the bone above the sinus to eliminate diploic anastomoses (see below)

The pO_2 and pCO electrodes used in the present experiments were both designed for application onto the exposed pia arachnoidal membrane the active surface of each electrode covering a cortical area 2 to 4 mm in diameter The reasons for such a design were several the two to most important being the hazards introduced by even discrete surface damage to the tissue (SIESJO 1961) and the variations encountered (in tissue pO_2) when measuring on different cortical locations with electrodes of smaller size (DAVIES and BRONK 1957) These latter variations evidently were influenced by the presence of surface vessels The pCO_2 measured with electrodes of the present size, however is only influenced by the largest surface veins (SIESJO 1961) These were therefore avoided in the present experiments

The pO_2 electrode (LUBBERS 1960) was a recessed type of the membrane covered platinum electrode (*cf* DAVIES and BRINK 1942) The recess formed a chamber filled with saturated potassium chloride The chamber was 400 μ deep and had lateral walls, 1 mm thick, to exclude any influence of the oxygen pressure of the surrounding medium upon the measurements The electrode was operated intermittently so that when the recess was allowed to equilibrate with the tissue oxygen, no current was drawn At the end of the equilibration time (10—15 minutes) a negative potential of —0 5 to —0 7

volt was applied to the cathode current being drawn for 3 seconds The readings were made with a Nanoamperemeter (SIEMENS) During the equilibration time the animal was closely observed to ensure constancy of respiratory and circulatory conditions as well as of cortical functional activity (EEG)

The pCO_2 electrode (HERTZ and SIESJO 1959 SIESJO 1961) was assembled with 0 001 N $NaHCO_3$ and with 12 μ Teflon The electrode was suspended in a needle bearing so as to exert minimal pressure onto the tissue surface. The signals from the electrode were amplified by a pH meter (PHM 22 RADIOMETER, Copenhagen) and fed to one of the DC-channels of an OFFNER type D3 Electroencephalograph and recorded on paper simultaneously with the EEG and the blood pressure Absolute readings of tissue pCO_2 were taken from the pH meter the same meter being used both for measurements and for calibrations.

The pCO_2 values obtained on the arachnoidal surface of anaesthetized animals do not vary significantly between different cortical areas (SIESJO 1961) Under the conditions of the present experiments the placement of the pCO_2 electrode was therefore not considered critical The electrodes was thus placed somewhat behind the pO_2 electrode in a separate burr hole (*cf* Diagram 1 GLEICHMANN *et al* 1962)

Both electrodes were calibrated in gas streams at the temperature of the cortical surface which was measured subdurally close to one of the electrodes The temperature measured at the cortical surface was usually somewhat lower than that in the oesophagus (*cf* Table I GLEICHMANN *et al* 1962) The gas tensions in the blood samples and the values obtained at the tissue surface were thus determined at slightly different temperatures

Prior to the experiments described here a series of cortical pCO measurements were made without previous elimination of the diploic anastomoses to the superior sagittal sinus The pCO_2 values obtained in that series often differed substantially from the calculated average tissue pCO_2 and often came close to the pCO measured in sinus blood It was also found that the pCO_2 of the cortical venous blood samples varied with the rate of aspiration from the sinus cannula On the assumption that the aspiration caused a varying degree of contamination with extracerebral blood via the diploic anastomoses these anastomoses were carefully eliminated in all subsequent experiments by means of a longitudinal craniotomy The speed of blood sampling from the sinus was however also adjusted to the normal rate of outflow from the cannula since too rapid a sampling often gave a small decrease on the continuous record of pCO_2 from the cortical surface (1 to 2 mm Hg) When such a decrease occurred the pCO obtained immediately before and after the decrease was taken as the actual tissue pCO level

After the craniotomy the dura over the measuring sites was left intact until the sinus had been cannulated and the animal had remained for some time at a reasonably steady state with regard to blood pressure respiration and EEG The dura was then opened care being taken not to damage the cortical surface and the electrodes were brought into place The analyses were postponed until temperature equilibrium was achieved between the electrodes and the cortical surface When the electrodes were removed for calibration the cortical surface was protected from drying by application of cotton wool soaked in body warm Ringer s solution

Results

I *The pO_2 measurements*

The results of the pO_2 measurements are shown in Table I For each individual value of $rCMR_O$ the Δ pO_2 was calculated and subtracted from the mean capillary tension The resulting calculated average tissue oxygen tensions are

Table 1 Cerebral cortical pO_2 The $rCMR_O$ values were obtained from the regional cortical blood flow and the arteriovenous oxygen differences (GLEICHMANN *et al* 1962) *The ΔpO_2 values were obtained by multiplying the individual $rCMR_{O_2}$ values with the appropriate constant (see Methods A) When the ΔpO_2 values were subtracted from the mean capillary pO_2 the calculated average tissue pO_2 was obtained ($\bar{p}_tO_2$) When a mean $rCMR_{O_2}$ of 7 0 (ml O_2/100 g/min.) was used for the calculation of ΔpO_2 another average tissue pO_2 resulted ($\bar{\bar{p}}_tO$ b) The differences between the pO_2 values obtained with the electrode on the pia arachnoidal membrane (p_tO_2 meas) and the two sets of calculated average tissue pO_2 are given in the last column. It is seen that there is a fair agreement between the pO_2 value obtained with the electrode and that calculated on the basis of a uniform $rCMR_O$ The details of the experiments (denoted by numbers in column 1) are accounted for in table I of* GLEICHMANN *et al 1962*

Exp.	pO mm Hg			$rCMR_{O_2}$ mC/100 g /min.	ΔpO_2 mm Hg	$\bar{p}_tO$ a calc. mm Hg	p_tO_2 meas. mm Hg	$\bar{\bar{p}}_tO$ b calc. mm Hg	Diff. p_tO_2 meas. — $\bar{p}_tO$ calc. mm Hg
	a. femoral.	s sag sinus	mean capill.						
VI 3	75 0	45.5	57 0	4 0	10.5	46 5	38.5	39 0	a — 8 0 b — 0.5
5	80.5	44 5	57 0	5 5	14.5	42.5	39 5	39 0	a — 3 0 b + 0.5
VIII 4	81 0	41.5	60 0	8 6	22.5	37.5	42 0	42.0	a + 4.5 b ± 0 0
IX 1	98.0	26 5	42 5	7 1	18 5	24 0	25 0	24.5	a + 1 0 b — 0 5
2	98 0	27 0	41 0	9 7	25 5	15.5	27 0	23 0	a + 11.5 b + 4 0
3	58 0	25 5	43 0	12 1	31 5	11 5	27 0	25 0	a + 15 5 b + 2.0
4	113 0	20 5	35 0	13 4	35 0	0 0	18.5	17 0	a + 18.5 b + 1 5
5	50.5	19.5	25 5	4 9	13 0	12.5	9 0	7 5	a — 3 5 b + 1.5

given in the column to the left of that denoting the individual electrode measurements of pO_2 In the next column are given the calculated average tissue oxygen tensions obtained with an average value for $rCMR_O$ of 7 0 ml/100 g/min (GLEICMANN *et al* 1962)

The tissue pO_2 measurements are too few to permit a statistical analysis. Some facts may however be pointed out First the pO_2 measured with the electrode differs significantly from the mean capillary tension in all eight analyses Second in most cases the pO_2 directly measured comes close to the pO_2 in venous blood Third if the pO_2 measured is compared with the average tissue pO_2 calculated from the individual values of $rCMR_O$ a fair agreement is obtained in only half the cases If however the comparison is made with an

Table II Cerebral cortical pCO_2 The $rCMR_{CO_2}$ values were calculated from the regional blood flow and the arteriovenous carbon dioxide differences The ΔpCO_2 values were obtained by multiplying $rCMR_{CO_2}$ with the appropriate constant (see Methods 4) The ΔpCO_2 represents the difference between the mean capillary pCO_2 and the calculated average tissue pCO_2 ($\bar{\bar{p}}_tCO_2$ calc) $\bar{\bar{p}}_tCO_2$ meas is the pCO_2 value obtained with the electrode at th pia-arachnoidal membrane Note simi larity between the values of pCO_2 measured and calculated respectively The details of the experiments (denoted by numbers in column 1) are accounted for in table I of GLEICHMANN *et al 1961*

Exp		pCO mmHg			rCMRco CO ml/100g/ min	Δ pCO_2 mm Hg	$\bar{\bar{P}}_t$CO calc mm Hg	P_tCO meas. mm Hg	P_tCO_2— p_tCO mm Hg
		a femo- ral.	s. sag s nus	mean capill.					
II	1	26 0	54 0	40 0	6 0	0 9	40 9	42 5	+ 1 6
IV	1	29 0	51 0	40 0	6 2	0 9	40.9	40 0	— 0 9
	2	29 5	49 5	39 5	4 6	0 7	40 2	39 0	— 1 ?
	3	27 5	49 5	38.5	5 1	0 7	39 2	40 0	+ 0 8
	4	30 0	59 0	44 5	2 5	0 4	44 9	45 0	+ 0 1
V	1	30.5	50 5	40 5	7 0	1 0	41.5	41 0	— 0.5
	2	31 5	53 0	42.5	8 0	1.2	43 7	42 0	— 1 7
	3	43 0	69 0	56 0	6 9	1 0	57 0	54 0	— 3 0
	4	22 0	40 0	31 0	6.6	1 0	32 0	30 5	— 1 5
VI	4	40 5	53 5	47 0	5 7	0 8	47 8	49 0	+ 1 2
VIII	1	0 0	50 0	40 0	6 1	0 9	40 9	41 5	+ 0 6
	2	30 5	61 0	45 5	5 6	0 8	46 3	46 0	— 0 3
	3	44 5	54 0	49 0	11 8	1 7	50 7	47 0	— 3 7
IX	3	30 0	51 5	40 5	11 9	1 7	42 2	40 5	— 1 7
	4	14 0	28 0	21 0	13 7	2 0	23 0	26 5	+ 3.5
	5	52 0	63 0	57 5	3 7	0 5	58 0	57 0	— 1 0
X	1	32 0	56 0	44 0	7 0	1 0	45 0	42 0	— 3 0
	2	26 5	56 0	41 5	7 0	1 0	42 5	45 0	+ 2 5
	3	31 5	45 5	38 5	7 0	1 0	39.5	40 5	+ 1 0
XI	1	37 5	56 5	47 0	7 0	1 0	48 0	56 5	+ 8 5
XIII	3	28 5	46 5	37 5	7 0	1 0	38 5	41 5	+ 3 0
	4	34 5	50 0	42.5	7 0	1 0	43 5	44 5	+ 1 0
	5	25 0	43 0	34 0	7 0	1 0	35 0	34 5	— 0 5

average tissue pO_2 calculated from the average $rCMR_{O}$ value of 7 0 ml/100 g/ min a fair agreement is reached in all analyses

II *The pCO_2 measurements*

The results of the pCO_2 measurements are shown in Table 2 There were twenty three successful measurements In the first seven of these (experiments X XI and XIII) no measurements of rCBF were carried out. For this series a $rCMR_{CO}$ of 7 0 ml/100 g/min (GLEICHMANN *et al* 1962) has been assumed

giving a Δ pCO_2 of 1 mm Hg The assumption cannot introduce any large error since the influence of $rCMR_{CO}$ upon Δ pCO_2 is small The series is also rather homogenous with regard to the pCO_2 in arterial and venous blood

In the results there are two measurements which were not carried out at steady state (IX 4 and XI 1) They were included in the group as they are of some interest to the interpretation of the values (see Discussion) Fifteen to twenty minutes before each of these measurements the respiratory minute volume was increased and the pCO_2 recorded from the cortical surface was still decreasing at the time of the blood sampling These values will be discussed sparately

A statistical analysis was carried out so as to correlate, on the one hand, the pCO_2 measured with the electrode and on the other, the calculated average tissue pCO_2 the mean capillary pCO_2 and the arterial and cortical venous pCO_2 The differences of the arithmethic means of the first three of the above groups were also calculated together with a standard deviation of the mean

It is seen that the pCO_2 values obtained with the electrode lies *above* the middle capillary tension (+ 0 7 mm Hg), but *below* the calculated average tissue pCO_2 (— 0 3 mm Hg) the standard deviation from the mean being almost identical (± 1 1 mm Hg and ± 1 0 mm Hg respectively) A very high correlation was found between the pCO_2 measured on the cortical surface and (1) the average tissue pCO_2 calculated (coefficient of variation r = 0 97), as well as (2) the middle capillary tension (r = 0 97) the correlation towards (3) the cortical venous pCO_2 (r = 0 88) and towards (4) the arterial pCO_2 (r = 0 87) being somewhat lower though still highly significant

Discussion

As indicated in the introduction measurements of the tissue oxygen and carbon dioxide tensions would be of much help in an experimental approach to many problems pertaining to the function of the cerebral cortex Until recently only qualitative measurements of tissue oxygen tension have been performed Such measurements are however in most cases of very restricted value In principle quantitative measurements have been possible with the recessed type of platinum electrode (DAVIES and BRINK 1942 DAVIES and BRONK 1957) The application of such electrode measurements the biological problems have however been hampered by the difficulty of interpreting the tissue tensions measured Thus different tensions have been measured on different loci of the cortical surface depending upon the distance to surface vessels (DAVIES and BRONK 1957) Consequently the above interpretation must be regarded a prerequisite for quantitative measurements on a tissue where tension gradients are present due to diffusion processes

The electrodes used in the present investigation were both deliberately devised so as to cover a relatively large cortical area (diameter of active areas ?

to 4 mm) It was thought that such a design would make the measurements independent of tension gradients around superficial vessels and possibly also make the tension measured come close to an average tissue tension The first of these assumptions was strengthened by the pCO_2 measurements cited above the second remained the object of the present experiments

As a consequence of the design of the electrodes the measurements had to be confined to the surface of the tissue This must not however be regarded a disadvantage but rather a prerequisite Thus the insertion of electrodes into the tissue will inevitably give rise to damage the effect of which upon the measuring conditions cannot be predicted (*cf* SIESJÖ 1961) From a quantitative standpoint the insertion of electrodes into the tissue will only make sense if the electrodes can be brought down to such a size (a few microns) that intercapillary tissue regions can be explored

The theoretical considerations involved in the present investigation are based on certain assumptions Thus, the venous blood samples must be considered representative of a large cortical area Evidently if the comparison attempted shall be justified the measurements of rCBF as well as of tissue pO_2 and pCO_2 must also represent the same area There are good reasons to believe that rCBF in anesthesia is rather uniform between different locations in the part or the cortex under discussion (*cf* INGVAR and LASSEN 1962) As for the pCO_2 very small differences have been found between different cortical regions (see above) and the same seems to be true also for pO_2 at least in symmetrical cortical regions (KUNZE GANSHIRT and LUBBERS 1962)

Any correlation of the pO_2 or the pCO measured at the surface of the tissue with the average tension in the tissue must take into consideration the approximations involved in the calculation of the latter For a discussion of the calculation reference is made to the original papers (THEWS 1960 a and b) The average tissue tension is dependent upon two main factors the mean capillary tension and the Δ p resulting from the consumption of oxygen and the production of carbon dioxide respectively Since the involved factors differ quantitatively for the two gases they are treated separately

pO_2 In the calculation of the mean tension from the tensions in arterial and cortical venous blood the nonlinearity of the oxygen dissociation curve has to be taken into account Moreover the Δ pO_2 (representing the decrease in pO_2 due to the oxygen consumption of the tissue) has a relatively large value (compared to the Δ pCO_2) on account of the low oxygen diffusion coefficient This means that experimental as well as theoretical errors may cause larger absolute variations between the tension calculated and the one measured (*cf* Table I)

pCO_2 The calculation of the average tissue pCO_2 is simpler First the physiological carbon dioxide dissociation curve can be treated as a straight line (Fig 1 *cf* also CHRISTIANSEN DOUGLAS and HALDANE 1914) which means that the mean capillary tension is the simple arithmetic mean of the tensions in the arterial and the venous blood Second due to the high diffusion coefficient for

carbon dioxide in the tissue, the Δ p is small (1 mm Hg at a $rCMR_{CO}$ of 7 0 ml/100 g/min) On the other hand the similarity between the mean capillary tension and the average tissue tension will make it hard to distinguish between them

The following facts seem especially evident from the present results First, the pO_2 obtained with the electrode lies close to the cortical venous pO_2 but far from the average capillary pO_2 Second, the pCO_2 measured with the electrode lies close to the mean capillary tension but far from the venous pCO_2 Provided the conditions determining the tensions measured with the two electrodes are identical these results indicate that both electrodes measure a tissue tension and neither a mean capillary nor a venous tension

Further conclusions must, however be drawn with caution The pO_2 measurements are too few to permit positive statements The results clearly indicate that the electrode does not measure a mean capillary pO_2 The question whether the electrode measures a tension close to the calculated average tissue pO_2 must however be left open The resemblance between the tissue oxygen tensions measured and those calculated on the basis of an average $rCMR_0$ suggests one of three possibilities 1) the pO_2 electrode does not measure the average tissue pO_2 2) the determination of $rCMR_0$ gives erroneous results Both of these possibilities could be true if the different methods involved did not implicit the same tissue compartments 3) The approximations and assumptions involved in the calculation give erroneous results Until further experiments are available no statement can at present be made as to the cause of the discrepancies encountered

The results of the pCO mesurements are more simple to interprete Thus the highest correlation is obtained towards the mean capillary tension and towards the calculated average tissue pCO_2 but on account of the similarity between these tensions it is very hard to state if the tension measured with the electrode is closer to the one or the other Three facts suggest that the calculated average tissue pCO_2 is the one measured First the arithmetic mean of all measurements are closer to the calculated tension than to the mean capillary tension Second the pO_2 measurements indicate that electrodes of the present size do not measure the mean capillary tension (ee above) Third in a non steady state after a change in ventilation (see results) the pCO_2 values obtained with the electrode differ unduly from the calculated average pCO_2 This difference which is most likely explained by a lag in the carbon dioxide diffusion from the tissue cannot be explained by the response time of the electrode, all the more as it failed to occur in the same time period after induced hypoventilation

On rather strong grounds it can thus be assumed that a pCO_2 electrode of the present type measures a tension that is close to the actual average tissue tension provided that the assumptions and approximations made in the calculation are justified It must however be born in mind that neither the

electrode method nor the calculation can produce strict evidence ad to the nature of the tension obtained with the other method The resemblance in results with the two methods are however suggestive and, as long as no other method of comparison is available, we might assume that a surface pCO_2 electrode of the type and size used here does measure the actual average tissue pCO_2 of the cerebral cortex

Note added in proof The ΔpCO_2 values in Table II were calculated from a preliminary value for the diffusion coefficient for carbon dioxide $K_{CO} = 4\ 1$ ml/cm/min/atm) The final evaluation of this coefficient (SIESJO and THEWS 1962) has given the value 5 4 ml/cm/min/atm Correct values for ΔpCO_2 in Table II are then obtained by dividing each value with the factor 1 32 This alteration is significant only insofar as the mean difference between the measured and the calculated tensions respectively is reduced to about 0 1 mm Hg Consequently there is a more marked difference between the mean capillary tension and the measured tissue carbon dioxide tension than is obvius in the Table

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Acta physiol. scand. 1962 55 139—149

From the Department of Physiology University of Uppsala, Sweden

Effects of Two Rapid Acting Cardiac Glycosides on Dog's Heart-Lung Preparation

By

NILS-HOLGER ARESKOG

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Abstract

ARESKOG N H *Effects of two rapid acting cardiac glycosides on dog's heart lung preparation* Acta physiol scand 1962 *55* 139—149 — The effects of two rapid acting cardiac glycosides convallatoxin and acetyl strophanthidin, were investigated on heart lung preparations of dog The positive inotropic effect the electrolyte effect (induction of cellular potassium loss) and the toxic effect (induction of tachycardia and arrhythmia) were studied with special regard to magnitude and time course of response Changes of plasma pH in the HLP were caused by changes of P_{CO_2} in the inspired air A difference was found between convallatoxin and acetyl strophanthidin For acetyl strophanthidin the magnitudes of the electrolyte and the inotropic effects showed positive correlation in different preparations for convallatoxin the inotropic effect tended to decrease with an increasing electrolyte effect This difference in action between the glycosides supports the theory that the mechanisms for their inotropic and electrolyte effects are separate. Another difference between the two glycosides was that acetyl strophanthidin had an inotropic optimum at a lower plasma pH level than convallatoxin The result further suggests that the electrolyte and the toxic effects are related at least more so than the electrolyte and the inotropic effects

The cardiac glycosides have three main effects the positive inotropic action on failing myocardium which may be called the *therapeutic effect* the influence on the electrolyte balance of different kinds of cells (resulting in an increased potassium loss from the cells) which is usually called the *electrolyte effect* and the disturbance of impulse formation and conduction resulting in e g arrhythmia which as a rule is named the *toxic effect*

Whether or not the two first mentioned effects are causally interrelated has for several years been a matter of discussion CALHOUN and HARRISON (1931) and WOOD and MOE (1938) observed a potassium loss from isolated myocardium with digitalis therapy REGAN, TALMERS and HELLEMS (1956) found an increase in the plasma content of potassium after administration of acetyl strophanthidin in therapeutic doses Recently BLACKMON et al (1960) observed that both toxic and nontoxic doses of heart glycosides produced a rapid release of potassium from dog s myocardium On the other hand HAGEN (1939) observed a slight increase in the myocardial potassium concentration with therapeutic doses of digilanid but a marked decrease with toxic doses BOYER and POINDEXTER (1940) also found in dogs an increase in the intracellular potassium content of the myocardium after digitalization Still another view is represented by GONLUBOL SEIGEL and BING (1956) who did not find any significant change in the myocardial potassium balance after administration of cardiac glycosides in man

With regard to the divergency of opinion it seemed of interest to compare in detail the actions of different types of cardiac glycosides The problem is whether or not the inotropic effect has a fixed relationship to the other two main effects the magnitude of these effects and their time courses with different doses are taken as indices of the relationship

In the present report the two rapid acting cardiac glycosides *convallatoxin* and *acetyl strophanthidin* have been investigated on heart lung preparations (HLP) of dogs with regard to the intensity and time course of the differential actions The influence of changes of plasma potassium in the HLP on the glycoside action has also been investigated

Material and methods

The experiments were performed on mongrel dogs weighing 10—26 kg They were anaesthesized with Nembutal® administered intravenously in a dose of 30 mg/kg body weight To prevent clotting heparin in a dose of about 5 mg/kg body weight was injected before switching on to HLP Positive pressure respiration was performed by a Starling pump Changes of pH were induced by changing P_{CO_2} in the inspired gas mixture (2 7—6 5 % CO_2 in oxygen) After careful ligation of all vessels (it is especially important to ligate all small vessels passing from pericardium to mediastinum and thymus to get the system absolutely free from leakage) the brachiocephalic trunk on the arterial and the superior vena cava on the venous side were cannulated The arterial cannula was connected to a big Windkessel i e an air chamber to simulate the volume elastance of the larger arteries The arterial resistance and venous supply were kept constant during the experiment Recording of arterial and venous pressures was performed, using a strain gauge or a Wismut Tauchspulen Manometer according to HAMILL (1940—41) as transducers further heart rate temperature (kept constant by a thermostat) and in some cases ECG were recorded at frequent intervals A multichannel optical oscillograph with one slow and one fast camera was used Further description of the presently used HLP method will be published elsewhere (ARESKOG 1962)

By injecting the glycosides at a certain time after the start of HLP and by also standardizing the experiments with respect to arterial vascular resistance venous supply

and pulmonary ventilation it was possible to obtain heart preparations of similar degree of myocardial insufficiency and therefore comparable response to the glycosides As a HLP has a limited capacity to break down cardiac glycosides even small doses tend to become toxic due to repeated recirculation and therefore a high myocardial uptake

The potassium sodium and in some cases calcium concentrations of plasma were analyzed by flame photometry The measurements of pH in a Beckman Gs pH meter and in some cases Pco using a modified Severinghaus electrode according to GRANGSJO and ULFENDAHL (1958) (but instead of their rubber membrane a teflone membrane was used) were made on whole blood as soon as possible after taking the sample

Drugs Convallatoxin from *Convallaria majalis* (Convalyt Dr Madaus & Co Cologne) is known as the strongest of all hitherto discovered heart glycosides it was used in a dose of 0 005 mg/kg body weight (i e. the body weight of the intact dog) Acetyl strophanthidin (Lilly) is also a very rapidly acting glycoside and was used in a dose of 0 125 mg (one cat unit) per 15 kg body weight These doses caused toxic manifestations (severe arrhythmia) usually within 10—30 min after the injection to the HLP

Results

The inotropic effect of the cardiac glycosides

The increase of the arterial pressure has been taken as a measure of the positive inotropic effect This is justified as long as the venous supply and the arterial vascular resistance are kept constant

As seen in Fig 1 the maximum effect was reached about 10 min after the injection for both glycosides A secondary decrease towards the original level usually started after 15—20 min but in some cases a late rise of the arterial pressure was seen 20—30 min after the injection On an average the positive inotropic effect of acetyl strophanthidin was stronger than that of convallatoxin with the doses used The duration of the effect also seemed to be longer for acetyl strophanthidin The venous pressure was found to behave in inverse relationship to the arterial pressure acetyl strophanthidin had a more pronounced and prolonged effect than convallatoxin on venous pressure

In one experiment a very strong positive inotropic effect was recorded This heart was before the injection insufficient to an exceptional extent with a very low arterial pressure (60 mm Hg) and a very high venous pressure (7 9 mm Hg)

The electrolyte effect of the cardiac glycosides

Fig 2 shows the rate of potassium loss from the tissues of the HLP (i e the rate of increase of potassium in circulating plasma) It was rather constant during the first 20 min after the injection of the cardiac glycoside and of a similar magnitude for any particular dose and pH level in different preparations There was an increase at higher doses in parallel with an increased tendency to earlier appearance of arrhythmia When severe arrhythmia and especially when ventricular fibrillation occurred the rate of potassium loss increased considerably Acetyl strophanthidin initially caused the same or even a slightly higher rate of potassium loss than convallatoxin but after about 20 min

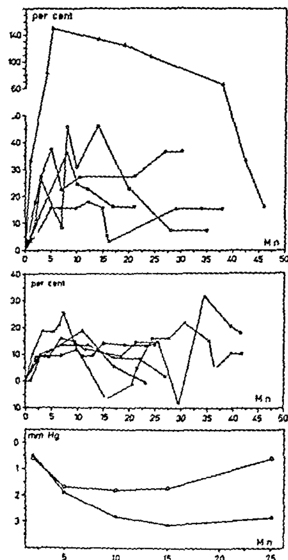
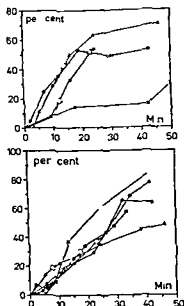


Fig 1 Percental increase of arterial pressure of *HLP* in relation to time after injection of acetyl strophanthidin (upper part pre injection range 60—110 mm Hg) and convallatoxin (middle part pre injection range 80—105 mm Hg) Different symbols denote different experiments Lower part shows average decrease of venous pressure after injection of acetyl strophanthidin (filled triangles) and convallatoxin (open circles) pre injection ranges for acetyl strophanthidin 3 3—7 9 mm Hg for convallatoxin 0 5—9 2 mm Hg Note that upper ordinates are alinear

the rate decreased more pronouncedly with acetyl strophanthidin than with convallatoxin

It is possible that the potassium increase of plasma was due to a loss from tissues other than the myocardial cells In control experiments it was found out that the potassium transport of the red corpuscles are not influenced by the glycoside dose used here A part of the potassium loss can be expected to occur in the lungs where according to WOOD and MOE (1942) more than a half of the potassium loss can take place However, in some experiments the lung circulation was disconnected for a time without any obvious signs of oxygen lack



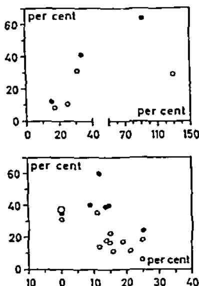


Fig 2 Fig 3

Fig 2 (Left) Percental increase of potassium concentration in plasma of HLP in relation to time after injection of acetyl strophanthidin (upper part, pre injection range 4 4—5 6 meq/l) and convallatoxin (lower part pre injection range 2 6—3 9 meq/L) Different symbols denote different experiments Appearance of arrhythmia is denoted by large dashed-open circles appearance of ventricular fibrillation by large open triangle

Fig 3 (Right) Percental increase of potassium in plasma (ordinates) after injection of acetyl strophanthidin (upper part) and convallatoxin (lower part) in relation to percental increase of arterial pressure (abscissa) values 10 min (open circles) and 25 min (closed circles) after injection of drug Pre injection ranges of arterial pressure for acetyl strophanthidin 60—110 mm Hg for convallatoxin 80—105 mm Hg of plasma potassium concentration for acetyl strophanthidin 4 4—5 6 meq/l for convallatoxin 2.6—5 0 meq/l Large circle denotes double dose of drug

during the glycoside action and a potassium increase in plasma from the working left ventricle was evident There is thus no doubt that the potassium loss also occurs in the myocardium but the values could not be used to calculate quantitative exchanges of potassium with any accuracy

Electrolyte effect related to inotropic effect

Fig 3 shows the ratio between the increase of potassium in plasma and the increase of the arterial pressure 10 min and 25 min after the injection of the cardiac glycoside While acetyl strophanthidine caused an increased plasma potassium most evident 25 min after the injection as well as an increased positive inotropic effect convallatoxin showed a decreased inotropic effect with increasing electrolyte effect Thus the two glycosides acted in opposite ways with regard to the ratio between the electrolyte and inotropic effects

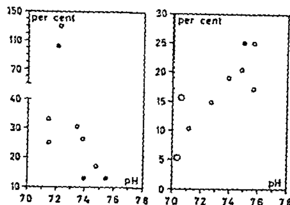


Fig 4 Percental increase of arterial pressure after injection of acetyl strophanthidin (left part) and convallatoxin (right part) in relation to plasma pH values 10 min (open circles) and 25 min (closed circles) after injection of drug Pre injection values of arterial pressure as in Fig 3 Large circles denote larger dose of drug Note that left ordinates are alinear

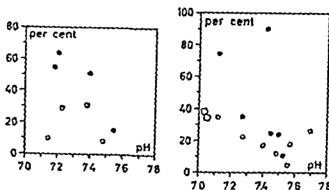


Fig 5 Percental increase of potassium concentration in plasma in relation to plasma pH after injection of acetyl strophanthidin (left part) and convallatoxin (right part) values 10 min (open circles) and 25 min (closed circles) after injection of drug Pre injection values of plasma potassium concentration for acetyl strophanthidin 4 4—5 6 meq/l for convallatoxin 3 4—5 0 meq/l large circles denote larger dose of drug

The positive inotropic effect of the cardiac glycosides and pH

In Fig 4 the increase of the arterial pressure in relation to pH is illustrated. There was also here a difference between the two glycosides Hypercapnia depressed the inotropic effect of convallatoxin but seemed to be without action on the inotropic effect produced by acetyl strophanthidin which has possibly an optimal pH level at a lower pH than convallatoxin

The electrolyte effect of cardiac glycosides and pH

As Fig 5 shows a hypercapnia (respiratory acidosis) tended to give a higher rate of potassium loss than was observed with normocapnia or moderate hypocapnia (respiratory alkalosis) At extreme hypocapnia the rate of potassium loss again tended to increase slightly Comparing the two glycosides in this regard there was no certain difference between convallatoxin and acetyl strophanthidin

Terminally when ventricular tachycardia or ventricular fibrillation occurred, the potassium loss increased markedly simultaneously with a decrease of pH in spite of the unaltered pulmonary ventilation and an unchanged or slightly

increased P_{CO_2} This indicates that the terminal acidosis depends mainly on metabolic factors

Sodium and calcium

The plasma sodium remained essentially unchanged after administration of both cardiac glycosides The average initial value was 144 meq/l the mean decrease was 0 7 % both 10 min (8 exp) and 25 min (7 exp) after the injection of the glycosides In some preparations there was a slight terminal decrease — not exceeding 3 % — of the sodium concentration but this effect was not significant (i e it was within the error of the method)

No change in plasma calcium was found during the action of the cardiac glycosides Assuming that the inotropic effect of the glycosides is dependent also on calcium ion distribution (LOEWI 1918 WILBRANDT 1955) this result could only be explained if a change occurs in the intracellular distribution of calcium (NIEDERGERKE 1957) without changes of total myocardial calcium content

Effects on heart rate impulse formation and conduction (toxic effects) of cardiac glycosides

There was either no or only a very small increase (a few per cent) of the heart rate related to the positive inotropic effect A more pronounced tachycardia appeared at the onset of arrhythmia This tachycardia was often not a sinus tachycardia but consisted of frequent extrasystoles in fixed coupling The arrhythmia did not seem to be associated with any particular value of potassium concentration or pH in plasma In a few experiments it was possible to restore sinus rhythm although a very high potassium loss had already occurred by injecting noradrenaline In some experiments toxic arrhythmia first appeared but later spontaneously decreased or ceased (although conduction disturbance remained) in spite of a high potassium loss

The arrhythmia depended on different types of disturbances of impulse formation and conduction e g atrio-ventricular block ectopic atrial or ventricular beats rapid ectopic atrial rhythm and terminally ventricular tachycardia or fibrillation

A potassium loss was found already before the onset of arrhythmia

Discussion

Usefulness of HLP in studying the actions of cardiac glycosides

Although it is useful to study the isolated heart in situ with the lungs as physiological oxygenators a disadvantage is that the electrolyte movements of the lungs and the heart cannot be separated Advantages are on the other hand that the HLP can easily be adjusted to a required pH level and that it is possible to get a rapid chemical equilibrium within the system

For a study of the inotropic effect of cardiac glycosides, failing or hypodynamic myocardium must be used as the contractile force of 'normodynamic' myocardium is not influenced by digitalis As was pointed out by REIN (1949) a HLP regularly develops a Spontaninsuffizienz i e progressive signs of myocardial failure and therefore should be suitable for such a study

As a HLP has a limited capacity to break down cardiac glycosides even small doses tend to become toxic but before the toxic effect appears there is, however, always a period of therapeutic effect of variable duration ("therapeutic phase of response)

The relationship between inotropic and electrolyte effect

HAJDU (1953) showed that the force of contraction and the spontaneous potassium loss of isolated frog hearts changed in parallel and further that potassium moved out from the cells during contraction and re entered during rest He suggested that the cardiac glycosides act by blocking the re entry of potassium thereby decreasing the intracellular ion (potassium) concentration

WILDE O BRIEN and BAY (1955) found evidence that with each cardiac cycle a potassium efflux occurred during repolarization Whether this efflux is enhanced by cardiac glycosides thus not only blocking the re entry but also increasing the efflux is still an unsolved problem

Most earlier reports on this problem have shown that toxic doses of cardiac glycosides give a potassium loss from myocardium but whether therapeutic doses also have this effect is disputed WOOD and MOE (1942) found a potassium loss from isolated dog s heart after therapeutic doses BLACKMON et al (1960) showed that both toxic and non toxic doses of cardiac glycosides produced a release of potassium from myocardium On the other hand GERTLER et al (1956) found no significant alteration in the potassium content of rabbit hearts after administration of digitoxin JOHNSON (1956) showed that glycosides with cardiac actions inhibited the net transport of potassium in the Sartorius muscle of frog while other glycosides with no or a very weak cardiac action did not inhibit the electrolyte transport He concludes that an influence on the potassium transport is necessary for the toxic effect of cardiac glycosides

In the present experiments a potassium loss occurred already within the therapeutic phase of action Although the time courses of the two effects were not similar this result may seem to support the view that a potassium loss is a pre requisite for the inotropic effect even of non toxic doses However the present doses of glycosides eventually became toxic and therefore the electrolyte effect may have been an early sign of toxicity the result can therefore not exclude the possibility that after all the inotropic and electrolyte effects are separate

The observed difference in behaviour between the two glycosides on the other hand supports the theory that the inotropic and the electrolyte effects are separate With convallatoxin the inotropic effect decreased with increasing

electrolyte effect with acetyl strophanthidin the two effects ran more in parallel The ratio between the inotropic and electrolyte effects was in most experiments higher for acetyl strophanthidin than for convallatoxin which means that a more pronounced electrolyte effect was seen with convallatoxin and a higher inotropic effect with acetyl strophanthidin at doses provoking toxic effects (arrhythmia) within a certain time range The inotropic and electrolyte effects of cardiac glycosides may therefore not have a direct causal relationship if indeed any relationship at all

Further support for the theory that the inotropic and electrolyte effects may be separate was obtained in HLP experiments with adrenaline noradrenaline and angiotensin (ARESKOG unpublished observations) A positive inotropic effect occurred after these substances without any change in plasma electrolyte concentrations This result shows that a positive inotropic effect can be obtained in the failing myocardium without any observable changes of plasma electro lyte concentrations It should be mentioned that cardiac glycosides have, in contrast to the above hormones the ability to produce a higher efficiency and a positive inotropic effect in HLP without a primary heart rate increase — in addition to the cardiac glycosides only Rein s spleen liver factor (SCHMIER 1956) shows this combination of effects The above mentioned hormonal effects therefore suggest but do not prove that the inotropic and electrolyte effects of cardiac glycosides also are separate

Even if the results presented here support the view that the inotropic and electrolyte effects are separate it is still possible that the inotropic effect may depend on intracellular electrolyte events e g a facilitation of the normal potassium loss during myocardial contraction which might improve the intra cellular ionic milieu for contraction

Relationship between electrolyte and toxic effects

In several earlier reports e g by WOOD and MOE (1942) and BLACKMON et al (1960) no obvious correlation between the *degree* of potassium loss and *appearance* of arrhythmia was found These results agree with the present results where in addition no correlation was found between the *rate* of potassium loss and *appearance* of arrhythmia However arrhythmia never appeared until a myocardial loss (i e increased plasma potassium concentration) was observed On the other hand it is well known that an excess of potassium in plasma before the injection of the cardiac glycoside prevents or delays the onset of arrhythmia This indicates that potassium movements in some way are of importance for the induction of arrhythmia

As has been mentioned earlier a potassium loss was always observed already before the onset of tachycardia and arrhythmia Thus is not in accord with the view that increased potassium loss is secondary to an increased heart rate The present results indicate that the potassium loss does not occur in parallel with the toxic effect i e the mechanism is not so simple that the increased potas-

sium loss is due to an increased number of contractions On the other hand some sort of relationship seems to exist and it seems probable that the electrolyte and toxic effects are more closely interrelated than the electrolyte and inotropic effects

Relationship between pH and action of glycosides

In the absence of drugs, respiratory acidosis causes a potassium loss in the intact animal organism. It has also been shown by HICKAM et al (1956) that respiratory alkalosis can give the same effect How pH influences the action of cardiac glycosides and conversely how cardiac glycosides influence the pH does not seem to have aroused much attention in the past. HELLEMS et al. (1953) found a hydrogen ion release in dogs injected with acetyl strophanthidin

With regard to the effect of the cardiac glycosides on potassium movements, it seemed to be of interest also to investigate the effect on hydrogen ion movements taking into consideration possible differences between the actions of acetyl strophanthidin and convallatoxin In the present experiments a respiratory acidosis increased the rate of potassium loss but a moderate respiratory alkalosis had no effect In this respect, there was no difference between the two glycosides but using the inotropic effect as an index acetyl strophanthidin showed an optimal pH range between moderate hypercapnea to normocapnea, while convallatoxin showed the highest inotropic responses with normocapnea to moderate hypocapnea

During the terminal arrhythmia the myocardial cells lost not only potassium but also hydrogen ions resulting in metabolic acidosis It seems probable that this depended on an increase of the intracellular hydrogen ion production this would in turn tend to compensate the potassium loss from the cells

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From the Department of Physiology Veterinarhogskolan, Stockholm 51 Sweden

The Effect of Electrical Stimulation of Nucleus Ruber on the Gamma Motor System

By

Bo Appelberg

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Abstract

Appelberg B *The effect of electrical stimulation of Nucleus ruber on the gamma motor system* Acta physiol. scand 1962 *55* 150—159 — In cats anesthetized with Nembutal electrical stimulation in the red nucleus caused inhibition of muscle spindle discharge in the contra lateral gastrocnemius and semitendinosus muscles With the aid of a sensitive myograph the inhibitory effect was shown not to depend upon variations in muscle tension. Recording from ventral root fibres revealed an inhibitory effect on gamma efferents Effective stimula ting points were histologically localized to the red nucleus It is con cluded that electrical stimulation of the red nucleus has an inhibitory effect upon the gamma motor system The function of the red nucleus as a link in a gamma controlling system including the precentral cortex, the globus pallidus and the red nucleus is discussed

Mechanisms for central control of the gamma motor system were demon strated by Granit and Kaada (1952) who found spots within the diencephalic and mesencephalic reticular formation which upon electrical stimulation yielded muscle spindle activation without altering muscle tension A facilita tory action on gamma efferents in the ventral roots and thus also on muscle spindles was also elicited from the hemispheric part of the anterior cerebellar cortex.

The above mentioned authors also found inhibitory areas in the bulboreti cular system and in the vermian part of the anterior cerebellar cortex.

The interesting problem of central gamma control was further studied by Eldred, Granit and Merton (1953) and by Eldred (1953) Several central structures were reported to influence the gamma system when electrically

stimulated Among other parts of the brain ELDRED also investigated the inter posite and dentate cerebellar nuclei and found these structures to affect the gamma system

As there is an intimate relationship between the interposite nucleus in the cerebellum and the red nucleus (APPELBERG 1961) it was considered interesting to study the effect of the red nucleus on the gamma system Moreover the final branches of the rubrospinal tract are known to end among the ventral horn cells in the spinal cord (COLLIER and BUZZARD 1901 HINMAN and CARPENTER 1959)

Evidence will be presented showing that the red nucleus despite being situated in the facilitatory reticular area of the mesencephalon has an inhibitory effect on the gamma system.

Methods

32 cats were used for the experiments They were anesthetized with intraperitoneally administered Nembutal (35 mg/kg) Small additional doses were given intravenously when needed

Three different ways of studying the gamma system and the central effects exerted on it were used

1 Muscle spindle activity in extensor (M gastrocnemius) and flexor (M semiten dinosus) muscles were recorded in thin dorsal root filaments In these experiments the fifth, sixth and seventh lumbar segments of the spinal cord were exposed The leg to be used was denervated except for the muscle from which the spindle activity was to be studied The leg was rigidly fixed with drills through the femur and the tibia A clamp was used to fix the ilium and another was fastened in the third or fourth lumbar spinal process. The tendon of insertion of the muscle to be used was cut and attached to a myograph. Pools of paraffin oil covered the exposed areas The oil was kept at a tem perature of 35—40 C by radiant heat.

2 The activity in gamma fibres was directly recorded from thin ventral root filaments. The operation was similar to that described above except that the whole leg was de nervated The tibial nerve was arranged for electrical stimulation thereby enabling reflex activation of alpha and gamma motoneurones

3 The activity in gamma fibres was recorded from thin strands of the cut nerve to the gastrocnemius muscle By electrical stimulation of the tibial nerve proximally in the leg the conduction velocity of the fibres recorded from could be determined

In all experiments a trephine hole was made in the roof of the skull contralaterally to the side recorded from in the cord or leg

After mounting the animal in the Horsley Clarke frame a rostrocaudal row of seven steel needle electrodes placed 1 mm apart was stereotaxically guided towards the red nucleus In another group of experiments a mediolateral row of five electrodes was used instead Electrical stimulation was performed between pairs of adjacent electrodes in the row The stimulus consisted of trains of square wave impulses (100—150 imp/sec pulse duration 1 msec)

The myograph used in the experiments with dorsal root recording was of the strain gauge type with a carrier frequency amplifier and demodulator The output of the myograph was connected to one of the two beams of the oscilloscope At maximum sensitivity a 1 gram load on the myograph spring gave a deflection of 9 mm of the beam.

Recording from the filaments was made differentially between two platinum electrodes. The impulses were displayed on one beam of the oscilloscope via an AC coupled amplifier. The second beam was used for the myograph or for the time marking.

At the end of the experiments the stimulating electrode sites were marked by electrocoagulations. The brains were perfused with Ringer's solution and with Bodian's fluid and embedded in celloidin. Sagittal or frontal sections (100 μ) were made through the pertinent region and the sections were stained with toluidin blue.

Results

I *Dorsal root records*

In these experiments muscle spindle activity as recorded in dorsal root filaments was used as an indicator of gamma activity. A certain level of spontaneous gamma discharge is always present in a good preparation. The rate of firing of a muscle spindle is dependent upon muscle tension and the activity in the gamma efferents controlling the spindle. As the muscle tension was kept constant in the experiments to be described, all changes in spindle frequency were considered to depend upon changes in the gamma activity.

Muscle spindle afferents were identified by their behaviour during a muscle twitch, i.e. by the pause in the discharge during the contraction phase (MATTHEWS 1933). In most cases this identification was confirmed by testing with the pinna reflex (GRANIT, JOB and KAADA 1952).

When it was thus considered verified that the activity from a single or a few muscle spindles was being recorded, the effect of central stimulation was tested. With the stimulating electrodes in the region of the red nucleus the stimulation resulted in a sudden decrease in the spindle discharge. This is illustrated in Fig. 1. In A is shown the characteristic pause during the muscular contraction. B and C are control sweeps showing the discharge at the muscle tension used. Small variations in spindle frequency were considered to be due to changes in the gamma activity as the myograph set at the highest sensitivity revealed no changes in muscle tension. D and E are consecutive sweeps in the beginning of the period of central stimulation showing a rapid decrease in the spindle discharge. No change is seen on the myograph. The low frequency is seen to be maintained during the whole stimulation period (F and G). H and I which were obtained immediately after cessation of the stimulation show that the original rate of firing is rapidly regained.

Effects of the type described were observed on 24 muscle spindle afferents in 15 different experiments. Muscle spindles in extensors (M. gastrocnemius) and flexors (M. semitendinosus) were equally influenced. The sensitive myograph used never revealed any muscle tension variations accompaning the changes in muscle spindle frequency.

It should be noted here that besides the 24 spindle afferents mentioned above 4 fibres were recorded from which in spite of having the characteristics of muscle spindle afferents were not influenced by the central stimulus. These fibres were, however, not further analyzed in the present investigation.



Fig 1

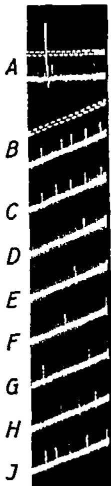


Fig 2

Fig 1 Records from a single muscle spindle afferent in dorsal root. Initial tension on muscle 31 g A. Single electrical shock applied to the muscle nerve causes a contraction of 60 g and a pause in the spindle discharge
B—I Consecutive sweeps at a rate of 1 5/sec showing the effect of electrical stimulation in the region of the red nucleus (note stimulating artefacts in D—G)
Myograph beam above each record (cf calibration besides I) Time each sweep comprises 250 msec

Fig 2 Records from thin ventral root filament
A Electrical stimulation of the cut tibial nerve causes reflex firing of a big alpha fibre. Note the spontaneously active small gamma fibre
B—I Consecutive sweeps at a rate of 1 5/sec showing inhibition of gamma discharge during electrical stimulation in the region of the red nucleus (note stimulating artefacts in C—F) Time 20 msec

2 *Ventral root records*

In order to eliminate the possibility that the effects observed were dependent upon small alpha driven variations in muscle tension a second series of nine experiments was made with recording from ventral root filaments

In such filaments small tonically active spikes easily influenced by pinching the pinna were identified as gamma fibres

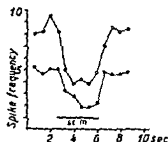


Fig 3 Diagram comparing the inhibitory effects on muscle spindles (open circles) and gamma efferents (filled circles) by electrical stimulation in the region of the red nucleus. For further description see text

It is well known, that while alpha fibres are but rarely spontaneously active in anesthetized animals an outstanding characteristic of gamma fibres is their spontaneous activity even in rather deeply anesthetized preparations (HUNT 1951)

HUNT also was of the opinion that gamma fibres may well be identified on the basis of spike height In the present series of investigations the alpha fibres were discharged reflexly by electrical stimulation of the cut ipsilateral tibial nerve Most filaments recorded from contained at least one alpha fibre besides one or a few gamma fibres

The effect of a pinna twist was used as the third criterion for gamma fibres GRANIT et al (1952) reported that pinna effects are regularly seen acting on gamma motoneurones in sufficiently active preparations

In the experiments with ventral root recording it very soon became apparent that there was an inhibitory effect on the gamma outflow when the region of the red nucleus was electrically stimulated All filaments recorded from acthe in a way similar to that illustrated in Fig 2 In Fig 2 A is shown that teh filament contained one large alpha fibre and one gamma fibre with a mucd smaller spike The gamma fibre was spontaneously active at a rate of about 18—20 impulses per second (B) During stimulation (C D E and F) the discharge decreased to 9 impulses per second.

In the diagram of Fig 3 is shown a comparison between the inhibitory effects as recorded on the afferent and efferent sides The two curves represent the mean frequency values from five muscle spindle afferents (open circles) and five gamma efferents (filled circles) It is evident that the time course of the effects in the two cases is principally the same

In the experiments with recording from ventral roots another observation was made In filaments which contained an alpha fibre it appeared that the reflex stimulation of this fibre was regularly either inhibited or facilitated from the same stimulating points which gave the gamma inhibition This is in agreement with the findings of SASAKI NAMIKAWA and HASHIRAMOTO (1960) These authors made intracellular records from alpha motoneurones They found that electrical stimulation in the red nucleus evoked an excitatory postsynaptic potential and spike discharge in contralateral flexor motoneurones and an inhibitory postsynaptic potential in extensor motoneurones



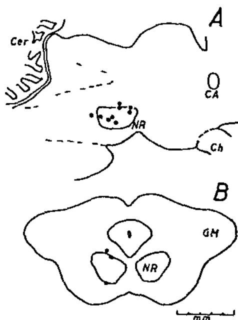


Fig 4 Records from a fine strand of the nerve to the gastrocnemius muscle
A Electrical stimulation of the tibial nerve 36 mm from the recording electrod The diphasic spike with a latency of 1 2 *msec is the gamma efferent* studied in the following sweeps Time 1 msec.
B—H Consecutive sweeps at a rate of 1 5/sec Electrical stimulation in the region of the red nucleus in D E and F (note stimulating artifacts) Slow waves are due to fibrillations in a denervated muscl Time 20 msec

Fig 5 Diagrams made by projection of sagittal (A) and frontal (B) serial sections through the region of the red nucleus In both diagrams is presented the maximal projection of the red nucleus in the two planes The black circles are electrocoagulations made in spots effective in causing gamma inhibition. The coagulation marks were projected down to the diagrams from histological sections from eighteen different experiments Abbreviations CA Commissura anterior Ch Chiasma opticum Ce Cerebellum GM Corpus geniculatum mediale NR Nucleus ruber

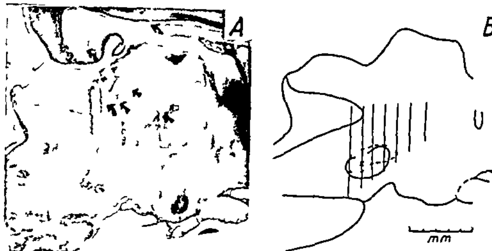


Fig 6 A. Sagittal section through the red nucleus showing stimulating electrode tracks and electrocoagulation.
B Diagram of the same section with all seven electrode tracks in the row drawn in their relative positions. The dashed line represents the outline of the area giving gamma inhibition The electrocoagulation (cross) was used as a reference point when measuring in the effective stimulating area.

3 *Nerve records*

In two experiments the spontaneous efferent activity in fine strands of the nerve to the gastrocnemius muscle was recorded By electrical stimulation proximally on the tibial nerve the conduction velocity of the fibres recorded from could be determined In these experiments it was clearly shown that the spontaneous activity in fibres with a conduction velocity within the gamma range (15—50 m/sec according to KUFFLER HUNT and QUILLIAM 1951) was inhibited by the central stimulus

In Fig 4 A is shown the response from a nerve filament on electrical stimulation 36 mm higher up on the tibial nerve The first part of the response is a big spike with a latency of less than half a millisecond A second smaller spike follows with a latency of 1 2 msec which means a conduction velocity of 30 m/sec This gamma fibre was spontaneously active as shown in B and C. By electrical stimulation in the region of the red nucleus it was inhibited in the same way as previously described for dorsal and ventral root filaments (D—H)

4 *Histological localization of stimulating points*

In the majority of successful experiments an electrocoagulation was placed in a spot which had the above described inhibitory effect on the gamma system Fig 5 A and B shows the localization of such electrocoagulations in eighteen

different experiments Though the diagrams are somewhat schematic in the sense that they present the maximal projection of the red nucleus in the sagittal (A) and frontal (B) planes it is apparent that all the lesions are situated in or very close to the red nucleus That a few of the lesions were actually found outside the red nucleus is probably explained by current spread to the nucleus in these experiments

In some experiments the whole area in the plane of the electrode row was carefully investigated by moving the electrodes up and down in 0 5 mm steps and testing the effect of electrical stimulation between different pairs of electrodes At the end of such experiments an electrocoagulation was made This coagulation was then used as a reference point when measuring in the area yielding the inhibitory effect This was made on a diagram of the histological section in which the electrode tracks were found Fig 6 presents the result of such an experiment The section with the centre of the electrocoagulation is shown above (A) Faint traces of six other tracks are also seen (arrows) These tracks were found in their whole length in nearby sections Fig 6 B is a diagram of the same section with all the seven tracks in the row drawn in their full length The dashed line encloses the area from which gamma inhibition was obtained in this experiment This area closely corresponds to the position of the red nucleus the outline of which is drawn in full

Discussion

The experimental findings described above prove that electrical stimulation of the red nucleus exerts an inhibitory effect on the gamma motoneurones The observation of a small number of muscle spindle afferents which were uninfluenced by the central stimulus does not alter this fact These spindles may have been damaged or their efferent gamma supply broken during the operation The possibility that there may exist muscle spindles which are not regulated from the red nucleus cannot however be excluded

Our present knowledge about the red nucleus and its connections with other central structures is still far from being complete It may therefore seem premature to try to draw any conclusions about the role played by the red nucleus in the central mechanisms controlling peripheral motor performance There are however certain experimental facts which may be used in order to throw some light upon the functional significance of the red nucleus

In spite of the intimate relationship between the interposite nucleus in the cerebellum which ELDRED (1953) found to influence gamma activity and the red nucleus (APPELBERG 1961) it does not seem likely that the red nucleus is a link in the cerebellar gamma inhibiting system studied by GRANIT and KAADA (1952) These authors obtained gamma inhibition by electrical stimulation of the vermal part of the cerebellar cortex This effect was also seen in

the decerebrate animal, and such a preparation is likely to have its red nuclei destroyed. The cerebellum may well exert its gamma regulation via bulbo-reticular pathways. SNIDER, McCULLOCH and MAGOUN (1949) described for example a cerebello — bulbo — reticular pathway for suppression of reflexes.

It seems more probable, that the effects on alpha motoneurones exerted by the red nucleus (SASAKI et al. 1960) are regulated from the cerebellum. POMPEIANO (1958) noticed that electrical stimulation of a paravermian strip of the cerebellar cortex caused an ipsilateral active flexion of fore- and hindlegs in the precollicularly decerebrated cat. Such a preparation has its red nuclei intact. A similar but contralateral effect was obtained by localized electrical stimulation in the red nucleus (POMPEIANO 1957 as quoted from DOW and MORUZZI 1958). It was further shown by POMPEIANO (1958) that the motor effects exerted from the cerebellar cortex could be abolished either by destruction of the interposite nucleus or by destroying the contralateral red nucleus.

At the present stage it therefore seems most reasonable to assume that the red nucleus on the one hand is a link in a cerebellar system influencing alpha motoneurones. On the other hand the red nucleus is also a component in a gamma controlling system. In this respect the red nucleus is probably under influence from cerebral centers. ELDRED et al. (1953) thus noticed inhibitory gamma influence from the contralateral internal capsule.

Though the relations between higher cerebral centers and the red nucleus are not fully investigated, there seem to be proofs enough for believing that such relations exist. Degeneration in the red nucleus was observed by METTLER (1947) after destruction of parts of the precentral cortex. LAURSSEN (1955) found degenerated fibres in the red nucleus after electrolytic lesions in the medial part of the globus pallidus. JOHNSON and CLEMENTE (1959) considered their material of degeneration studies after localized lesions in the basal ganglia to fully prove that direct connections exist between the globus pallidus and the red nucleus. MASSION (1961) demonstrated with electrophysiological methods focal potentials evoked in the red nucleus on electrical stimulation of the sensory motor cortex.

It therefore seems probable that when ELDRED et al. (1953) obtained inhibitory effects from the internal capsule, they were stimulating fibres passing from the cortex via the globus pallidus to the red nucleus.

It is tempting to assume that the two strikingly different cell types known to exist within the red nucleus are involved separately in the two functions of the nucleus.

The two types of cells are, however, not well separated within the nucleus. It does not seem possible, therefore, with the relatively crude stimulating technique used in the present series of experiments to selectively stimulate the one type of cells or the other. The problem of different cell type function must therefore be attacked with some other experimental methods.

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Arctic Aeromedical Laboratory — APO 731 Seattle Washington U S A

Heat Exchange Through the Muskrat Tail Evidence for Vasodilator Nerves to the Skin

By

Kjell Johansen*

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Abstract

Johansen K *Heat exchange through the muskrat tail Evidence for vaso dilator nerves to the skin* Acta physiol scand 1962 *55* 160—169 — The importance of peripheral vascular adjustments in the regulation of thermal balance has been studied in 25 adult muskrats This animal has a pelage of high insulative value while the tail is naked The importance of the naked tail for maintenance of thermal balance was studied by plethysmographic techniques and measurements of tail and rectal temperatures When subjecting the animal to a positive heat load by general body heating or following excercise the tail skin temperatures rose to 35—37 C Tail blood flow increased concomitantly by a factor of more than 400 The vasodilatation and subsequent increase in tail blood flow prevented heat accumulation and increase in rectal temperature during the positive heat load Nerve block of the tail before heating or excercise precluded the described increase in tail blood flow and hyperthermia resulted Nerve block at maximum blood flow led to a prompt decrease in tail temperature and a rapid increase in rectal temperature It is concluded that the muskrat tail is an indispensible heat exchanger reducing excess heat from increased metabolism or general body heating The data indicate that the conspicious changes in tail blood flow are mediated through a sympathetic vasodilator mechanism

The importance of peripheral vascular adjustments in the regulation of body temperature has long been appreciated The large bulk of information supporting this idea has however been derived from a surprisingly small number of species The arctic mammal offers in many ways a unique subject for research along these lines The pelage of these mammals has so high insulative value that no increase in metabolism is needed in order to maintain body

* Present address Institute for Experimental Medical Research, Ullevål Hospital, Oslo, Norway

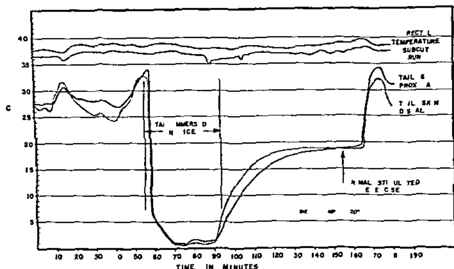


Fig 1 Rectal and subcutaneous temperatures on the trunk recorded simultaneously with intracutaneous temperatures proximally and distally in the tail Note the total vasoconstriction during the period of ice immersion and the sudden vasodilatation occurring shortly after the animal was stimulated to exercise

temperature even in air temperatures down to 50—60 C below zero (SCHOLANDER et al 1950) From the point of view of heat economy this fact poses the rather paradoxical question in an arctic situation How can the arctic mammal dissipate excess heat from increased metabolism during periods of excercise? Such heat dissipation occurs from the outer respiratory tract during panting in some species by sweating and by dissipation through the more sparsely insulated extremeties In the present study efforts have been made to evaluate the vascular changes in the tail for the heat economy of the muskrat *Ondathra zibethica*

Material and Methods

25 adult muskrats were trapped in the Fairbanks Alaska area during the months of September and October 1960 The animals were housed individually in wired cages. They were fed a fresh diet of lettuce and carrots and had access to water all the time

No general anesthesia or sedatives were used during the course of the experiments Starting an experiment the animal was transferred to a specially built small cage. The size of this cage was too small to allow for any major movements and the animal could not turn around in the cage The tail extended from the cage and could be tapered or tied down to an underlying board For measurement of blood flow changes in the tail two methods were used Direct and absolute values of blood flow through the tail were obtained by rubber strain gauge plethysmography measuring changes in circumference by recording resistance changes in the mercury within the rubber tube WHITNEY (1953) This method has recently been refined from its original by ELSNER EAGAN and ANDERSEN (1959) and by EAGAN (1961) Using the method on the muskrat

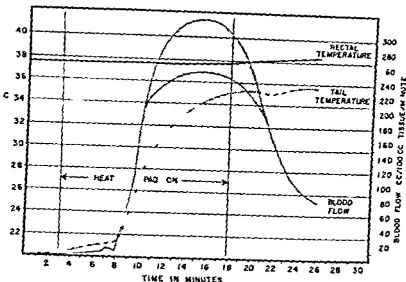


Fig 2 Changes in tail blood flow rectal and tail temperatures during a vasodilatation of the tail

tail the occlusion cuff was placed at the base of the tail and the gauge moved to different sites along the tail. Unless otherwise specified the flow has been measured at the base of the tail To get values on the relative changes in the tail blood flow intracutaneous temperatures were measured. Fine Cu K40 gauge thermo-couples were placed intra-cutaneously Deep rectal temperature was obtained with a thermo-coupe (gauge 30) placed 5—7 cm into the rectum. Subcutaneous temperatures on the trunk were obtained using implanted 40 gauge thermo-couples like for the tail temperatures.

the experiments with nerve blockade Novocaine Procaine or Xylocaine was used to infiltrate the tail at its base These drugs were guaranteed free from epinephrine. When subjecting the animals to a positive heat load they were either stimulated to excercise or a heat pad was put on the back of their trunk.

Results

Fig 1 shows rectal and subcutaneous temperatures on the trunk as well as intracutaneous temperatures proximally and distally in the tail At ambient temperatures around 20 the tail temperatures fluctuate between 20—35 These fluctuations occur spontaneously and very rapidly and are seemingly related to the role of the tail as a heat radiator A slight increase in core temperature is followed by a vasodilatation (flushing) of the tail and consequent heat loss Upon cooling of the tail in ice water as indicated on the figure the tail temperatures drop precipitously down to 1—2 The vasoconstriction prevents any loss of heat and the rectal and subcutaneous temperatures on the trunk show no decrease During the period of ice immersion there is no indication of a cold vasodilatation occuring in the tail In the post-cooling period when the tail is left in the ambient air the warming curve shows a typical exponential pattern indicating rewarming only by way of the external air

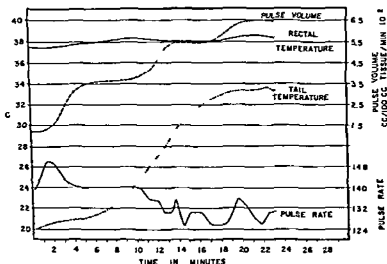


Fig 3 Pulse rate and pulse volume recorded simultaneously with rectal and tail temperatures during a vasodilatation or 'flushing' of the tail.

The fact that the most proximal and thinnest part warms most rapidly also substantiates this viewpoint At the point of the arrow the animal is stimulated to exercise The increase in heat production leads to a slight increase in rectal and subcutaneous temperature on the trunk The increased heat content of the animal is in turn followed promptly by a dramatic rise in tail temperatures causing an increased heat loss and a subsequent fall in core temperature In order to better evaluate the importance of the muskrat tail as a heat exchanger plethysmographic measurements of tail blood flow were undertaken In Fig 2 is shown the course of the tail blood flow expressed as ml blood per 100 ml of tissue per minute Rectal and tail temperatures are also depicted in the figure At the highest flows the error involved in the measurement increases due to the extremely rapid collection of blood upon occlusion of the cuff This increase in error is indicated in the figure by the shaded area In this experiment the capacious augmentation in tail blood flow occurs after the animal has been subjected to a positive heat load by putting a heat pad on its trunk During the heating period the tail blood flow increases by a factor of more than 400 This enormous increase in the blood flow takes place within a very short time 2—3 min In order to evaluate how this tail blood flow relates to the total cardiac output some measurements were undertaken using the thermodilution method for cardiac output (EVONUK 1961) These measurements were done on anesthetized animals which makes the comparison only suggestive The cardiac output of a series of muskrats of uniformly the same weight (600 g) showed an average of 225 ml/min To obtain maximal values of cardiac out puts adrenaline was infused into the sup vena cava This gave an increase to

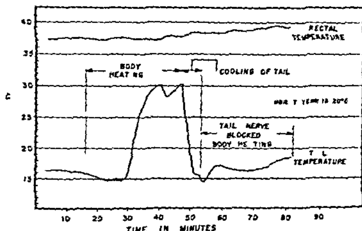


Fig 4 First portion of figure demonstrates the course of rectal and intracutaneous tail temperature during body heating and tail vasodilation in a normal undisturbed subject. Subsequently the tail is cooled down, the tail nerve blocked and body heating reapplied. Tail vasodilation and consequent heat loss is prevented and a rapid increase in rectal temperature is seen.

an average of 390 ml/min Comparing now the maximal blood flow to the tail with the maximum values obtained for cardiac outputs, the tail blood flow may represent approximately 10 % of the cardiac output The intracutaneous tail temperatures during these tail vasodilatations rose similarly in a very dramatic fashion reaching values from 0 5—3 0 lower than the deep core temperature

The effectiveness and physiological significance of the tail flushings as a means of losing excess heat were demonstrated in a number of ways. An occlusion cuff was placed around the basis of the tail. When the cuff was inflated to obstruct arterial inflow to the tail, the animal soon became severely hyperthermic after only short periods of exercise or in connection with an artificially produced positive heat load with a heating pad Some animals even died from heat apoplexia under such conditions Fig 3 shows changes in pulse rate and pulse volume in the tail as well as rectal and tail temperatures during one of the tail flushings Note the pulse rate changes insignificantly and if anything it drops slightly The pulse volume in the tail on the other hand increases by a factor of 4—5 times The increase shows a definite stepwise course

In order to investigate the possible mechanisms behind the conspicuous blood flow changes to the tail a series of experiments with nerve blockade was undertaken. 1 or 2 % Procaine Xylocaine or Novocaine all without adrenaline were used to infiltrate the tail at its basis Complete blockade was assumed present when all sensation in the tail was lost Fig 4 demostrates the course of events during such an experiment In the first half of the curve the increase in tail temperatures with the concomitant blood flow changes is shown in a

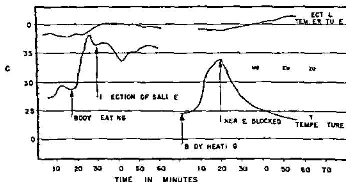


Fig 5 F rst portion of figure demonstrates the effect of injection of 2 ml saline at the base of the tail at maximum tail blood flow Last portion of figure demostrates the effect of a nerve block also performed at maximum tail blood flow Note the prompt vasoconstriction and increase in rectal temperature when the tail is nerve blocked

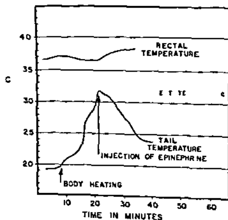


Fig 6 The figure demonstrates the p ompt asoconstricti e effect of injecting 5 g adrenaline in 2 ml saline at the base of the tail

normal undisturbed subject The increase in tail skin temperatures is provoked by body heating As can be seen the tail flushing is adequate in removing the excess heat and no or only slight increase in deep rectal tempetature is noticeable Next the heat load is removed and the tail is cooled down to temperature levels like before the start of the experiment Then the tail is nerve blocked and the body heating reapplied This time the tail temperature stays low and no vasodilatation occurs The animal has lost the crucial radiator surface of the tail skin and becomes rapidly hyperthermic This result is indicative of a *nervous* vasodilator mechanism to the skin of the muskrat tail Fig 5 demonstrates the effects of nerve blockade of the tail at peak blood flow The first part shows the injection of an equal volume of saline at peak flow ubsequent to the start of body heating in order to exclude the possibility of an obstruction of the blood

flow from the injection as a cause of the effect observed. The tail stays vasodilated at the high blood flow and heat is consequently removed at a rate preventing any increase in rectal temperature. Experiment 2 in Fig. 5 demonstrates a nerve block done at peak blood flow. This led to a sudden fall in blood flow eliminating the dissipation of heat from the tail skin. The rectal temperature shows a rapid rise and the animal soon gets severely hyperthermic. Fig. 6 demonstrates the effect of injecting small doses of adrenaline at the base of the tail. The prompt vasoconstriction illustrates the importance of performing the nerve block with anesthetics free from adrenaline.

Discussion

An effective radiator surface for the escape of heat constitute an integrative part in the heat exchanging systems in the homeotherm animal. In well furred arctic mammals the effective radiator surface is in most cases greatly reduced and constitutes in some cases only small areas on the snout and foot pads. SCHOLANDER et al. (1950 b), IRVING and KROG (1955), HAMMEL (1955), JOHANSEN (1961) have earlier shown the enormous insulative value of the fur in arctic mammals and noted (IRVING and KROG 1955) that sweating probably is of very rare occurence. Furthermore they never noticed any emanation of water vapor from the outer surface of arctic mammals. This fact renders the animals susceptible of heat accummulation during periods of intense muscular activity. The present study on the muskrat has demonstrated a vital role of the naked tail as a heat exchanger. Under the prevailing experimental conditions the intact tail even proved indispensible for the muskrat. Considering the very small surface occupied by the tail one can more easily appreciate the rather enormous changes in blood flow to the tail skin that are necessary in order to eliminate adequate amounts of heat. Thus the bare portions on the front and hindlegs plus the naked tail represent less than 5 % of the total weight of the animal and probably less than 10 % of the area effective as a radiator surface. The tail skin represents about 2/3 of the radiator surface available to the muskrat.

The blood flow changes to the tail as recorded in the present study seem to be without any parallel in other reports of similar nature. Thus the tail can show almost total vasoconstriction during a period of negative heat load (ice immersion) but shows flows 100 – 500 times greater shortly afterwards following a demand to dissipate heat. Under periods of maximal tail blood flow the data indicate that at least 10 [illegible] the total cardiac output is directed through the tail. Remembering that the tail represents less than 5 % of the total weight of the animal the size of the blood flow changes is further substantiated. It has some interest that Hos[illegible]o and L[illegible]ck (1957) assumed that maximum tail flow in the monkey (*Cercopithecus pygerythrus*) may represent around 10 % of the total cardiac output. They did not measure cardiac output but assumed values

similar to that of a dog of comparable weight The monkey they studied is a typical tropical species They concluded that the tail circulation is concerned in temperature regulation although they felt that their evidence for this function were disturbed by anesthesia

It seems conceivable that the reduction in effective radiator surface area, so common to most arctic mammals create a demand for a particularly effective control of the circulation to the remainder of the heat dissipating area In our concepts of vasomotor function the existance of vasoconstrictor and vasodilator nerve fibers are commonly recognised even though the idea still lingers that vasodilation is only effected by release of vasoconstrictor tonus Vasodilator fibres to skeletal muscle have been repeatedly reported and their existence confirmed (FOLKOW and UVNAS 1948 FOLKOW et al 1949 FOLKOW and GERNANDT 1952) for cats and dogs In man evidence of sympathetic vasodilator fibres to skeletal muscle have been presented by BARCROFT and EDHOLM (1945) There still remains considerable doubt about the existence of vasodilator nerves to the skin vessels From the point of view of heat economy one can appreciate great differences among various species in the importance of peripheral vascular mechanisms involved *in heat loss and conservation* The present findings show relevance to earlier studies on man only in so far as this is kept in mind The evidence brought forward in the present study seems conclusively to demonstrate the presence of vasodilator fibres in the skin of the muskrat tail The possibility of a vasodilation mediated through the formation of bradykinin from sweat gland activity (FOX and HILTON 1956) has been excluded. The tail skin was completely dry during the entire course of the experiments and no sweat glands could be detected histologically A remaining possibility was that a stable vasodilator substance were formed in the skin as a result of sympathetic activity during application of a positive heat load If the nerve block in this case was carried out prior to the heat load one would expect a prevention of the subsequent vasodilation If however the nerve block was performed at peak blood flow a prompt decrease like that obtained seems unlikely One would expect a more gradual depletion of the vasodilator substance produced prior to the nerve block.

In all the experiments abolition of the supposed vasodilator impulses was secured by nerve block of the appropriate nerve supply This procedure will naturally also remove all the nervous vasoconstrictor tone to the skin vessels If however the tail nerves are blocked at peak blood flow it seems likely that all or most of the vasoconstrictor activity has been removed Under these conditions the magnitude of the reduction in blood flow when applying the nerve block should be proportional to the activity of the vasodilator mechanism

It has been claimed several times that sympathetic vasodilator fibres are distributed to cutaneous areas in various species Thus BURN (1938) reported their presence in the ears of dogs On the other hand the presence of such fibres to any part of the skin in cats and dogs has been denied by FOLKOW and

Uvnäs (1948) and by Folkow et al (1949) In the human subject the existence of vasodilator fibres to the skin is still a matter of controversy The first evidence for their existence was obtained by Lewis and Pickering (1931) Their material consisted of patients suffering from Raynaud s disease Their finding was later confirmed by a number of workers (Fatherree and Allen 1938, Grant and Holling 1938 for the forearm skin Doupe, Cullen and Macaulay 1943) The latter authors furnished more direct evidence by reporting a dilatation of cutaneous vessels when stimulating sympathetic fibres to proximal parts of the human skin On the other hand, Sarnoff and Simeone (1947), Warren et al (1942) Arnott and Macfie (1948) and more recently Gaskell (1955) and Roddie Shepherd and Whelan (1957) have presented evidence against the existence of cutaneous vasodilator nerves in man

The present study suggests that nervous vasodilator activity to the skin vessels may exist as a specialized physiological mechanism It seems probable that such specializations may bear relevance to specific environmental conditions necessitating heat dissipation from particularly small portions of the skin

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Department of Physiological Chemistry, University of Lund Lund, Sweden

The Metabolism of 1-C^{14}-Palmitic Acid Labeled Chylomicrons in Rats

By

THOMAS OLIVECRONA

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Abstract

OLIVECRONA T *The metabolism of 1-C^{14} palmitic acid labeled chylomicrons in rats* Acta physiol. scand 1962 *55* 170—176 — 1 C^{14} palmitic acid labeled chylomicrons have been injected to carbohydrate fed rats and liver plasma, adipose tissue and carcass activities followed There was initially more activity going to the liver and adipose tissue and less to the carcass than when FFA was injected However although the mechanisms of uptake of FFA and chylomicrons differ fatty acids injected in either form soon enter the same pools and share the same fate No major difference could be found in the specific activities of samples from epididymal perirenal and subcutaneous adipose tissue

We have previously reported a study of the metabolism of 1 C^{14} palmitic acid injected as free fatty acid to rats (OLIVECRONA 1962) This paper describes a similar study of the metabolism of 1 C^{14} palmitic acid labeled chylomicrons which was done to enable a comparison of palmitic acid metabolism when injected in these differing states

Materials and Methods

100 μC 1-C^{14} palmitic acid (The Radiochemical Centre Amersham, England) was dissolved in a minute volume of ethanol mixed with 5 ml of cream and administered intragastrically to a rat on which a thoracic duct cannulation had been performed the previous day Chyle was collected for 24 hours in a flask chilled in ice. *It was layered* under 1 1 % sodium chloride solution and centrifuged at approximately 60 000 *g* for

Abbreviations used
TGFA — triglycerid fatty acids, NFFA — neutral fat fatty acids, FFA — free fatty acids, PLFA — phospholipid fatty acids.

Table I Composition of chylomicron preparation injected

	of total fatty acids	/ of total radioactiv ty
NFFA	89 7	92 9
FFA	2 6	0 2
PLFA	7 7	6 9

20 min The chylomicrons were sucked up into a syringe layered under 1 1 % sodium chloride recentrifuged and after being sucked up into a syringe again diluted to the desired volume with 0 9 % sodium chloride The composition and radioactivity distribu tion of the washed chylomicrons is shown in Table I The injected dose contained 50 000 cps and 120 mg fat in a volume of 1 0 ml

Due to the viscosity of the chylomicron emulsion the injection could not be carried out in a few seconds as is customary To standardize the procedure, the injection was carried out with constant rate for 2 min The time of 50 ° injection i e 1 min after the start of the injection was taken as zero time All other experimental procedures were identical with those previously described in a similar study of FFA metabolism (OLIVECRONA 1962)

Seven rats are included in the results presented The points in the curves thus represent values from single rats Similar experiments have been performed in this laboratory and have given essentially the same results However due to differences in the chylomicron preparations used it has not been possible to pool the results from different experiments

Results

The results are presented in essentially the same manner as previously de scribed in a similar study of FFA metabolism (OLIVECRONA 1962) Total ac tivities have been plotted not specific activities In this experiment systematic variation with time of the concentrations of fatty acids in some fractions oc curred This was most pronounced for plasma TCFA and PLFA which both decreased rapidly from initially high values towards normal values The liver TGFA rose to a maximum at 20 or 40 min then fell off towards normal values Liver PLFA and FFA did not vary systematically with time The values were 430 ± 70 and 12 8 ± 2 4 μeq/200 g body weight respectively and closely resembled those found in the FFA study (OLIVECRONA 1962) The plasma FFA concentration was fairly constant the value being 0 8 ± 0 3 μeq/ml.

Fig 1 shows the total activities in the plasma lipid fractions The initial decline of TGFA activity corresponded to a half life of approximately 10 min The initial half life of PLFA was approximately 23—29 min

Fig 2 shows the total activities per whole liver as percent of the adminis tered dose No correction has been introduced for the blood remaining in the liver Thus the early values probably are somewhat too high.

Fig 3 shows the body distribution of activity as percent of dose adminis tered Plasma and liver activities were calculated as previously described

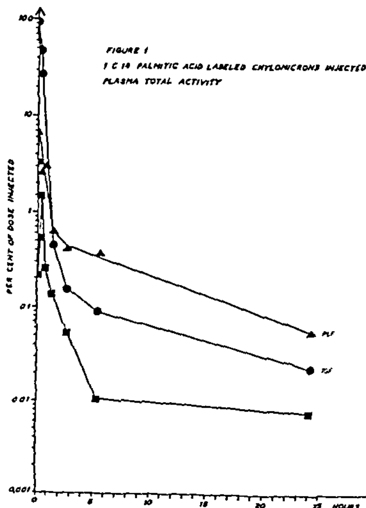


Fig. 1. Total activities of plasma lipid fractions after injection of 1-C[14] palmitic acid labeled chylomicrons into male rats.

(OLIVECRONA 1962). Carcass activity was calculated as previously described (OLIVECRONA 1962) but subtracted by the label in the plasma not taken out, calculated using the assumption that the plasma volume was 8 ml. The carcass value thus included adipose tissue but not the plasma. The total activity is the sum of carcass, liver and plasma activities calculated as above. The calculation of the adipose tissue activity was done as previously described (OLIVECRONA 1962).

Three different adipose tissues were studied. Their composition is shown in Table II and their specific activities in Table III. The composition was similar to that found in the FFA study (OLIVECRONA 1962). The specific

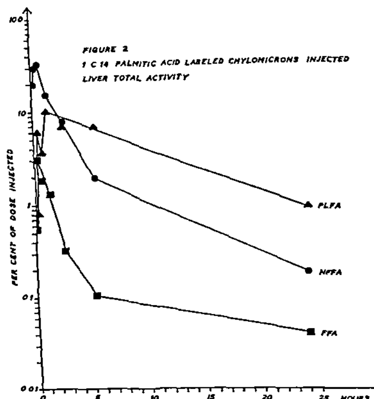


Fig 2 Total activities of liver lipid fractions after injection of 1 C palmitic acid labeled chylomicrons into male rats.

activities of the TGFA which was the fraction containing most of the activity in the perirenal adipose tissue tended to be higher than that of epididymal or subcutaneous adipose tissue TGFA The specific activities of the other fractions were more variable and no apparent trend could be seen These relations are similar to those found in a previous study of FFA metabolism (OLIVECRONA 1962) No major differences between the adipose tissues studied seem to exist as regards their capacity for uptake of chylomicron fatty acids

Table II Composition of different adipose tissues Fatty acids in the different fractions as of total fatty acids Values given are mean of 7 rats ± standard deviation of the samples

	Epididymal	Perirenal	Subcutaneous
FFA	0 26 ± 0 08	1 04 ± 0 32	0 44 ± 0 12
NFFA	99.2 ± 0 3	97 8 ± 0 7	98 7 ± 0 3
PLFA	0 51 ± 0.23	1 31 ± 0.28	0 84 ± 0 19

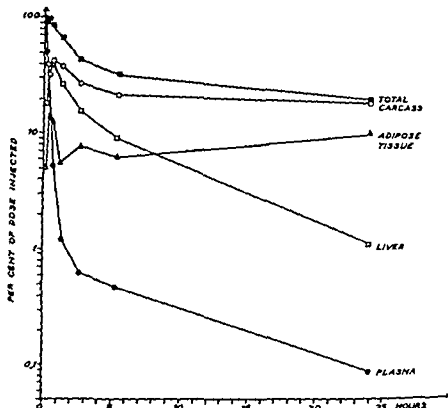


Fig 3 Tissue distribution of label after injection of 1 C palmitic acid labeled chylomicrons to male rats.

Discussion

The aim of this study was to compare the fate of 1 C^{14} palmitic acid injected as chylomicron fatty acid and as free fatty acid The experiment thus was carried out under similar conditions as those used in a previous study

Table III Specific activities in three different samples of rat adipose tissue after intravenous injection of 1 C^{14} palmitic acid labeled chylomicrons Values are given as cps per μeq of fatty acid Dose administered 50 000 cps 120 mg total fat per 200 g body weight

	Epididymal			Perirenal			Subcutaneous		
	FFA	NFFA	PLFA	FFA	NFFA	PLFA	FFA	NFFA	PLFA
10 min	8 1	0 032	1 ?	3 7	0 029	0.2	0 6	0 029	0 4
20 min	10 3	0 079	0 6	3 9	0 27	–	3.2	0 082	0 7
40 min	8 6	0 075	0 9	1 8	0 18	0 8	6 1	0 0;7	1.2
80 min	3 3	0 049	0 5	? 0	0 11	0 4	5 5	0 065	0 9
160 min	2 2	0 075	0 4	0 4	0 12	0 5	4 9	0 09;	0 6
6 hours	1 9	0 114	0 5	1 5	0 13	0 2	–	0 090	0 4
24 hours	1 4	0 059	0 3	0 3	0 10	0 3	–	0 0;8	0 4

of free palmitic acid metabolism (OLIVECRONA 1962) The dose of chylomicrons was very high and thus the present experiment was unphysiological However we thought that the high dose of chylomicrons might bring out the differences between chylomicron and free fatty acid metabolism more clearly as it would prolong the time of clearing of the chylomicrons and thus enable us to study more closely the tissue activity curves

BRAGDON and GORDON (1958) reported that the tissue distribution of chylomicron label was different from that of FFA label Cleared chylomicron activity in the fasting state was found primarily in the liver and after carbohydrate-feeding primarily in the depots FRENCH and MORRIS (1958) reported that in fasted rats the liver was the principal site of removal of chylomicrons Our own results are in good agreement with those of BRAGDON and GORDON and of FRENCH and MORRIS Figure 3 shows that the liver rapidly reached its maximal activity accounting for approximately 40 % of the injected dose After 40 min the liver activity rapidly declined again At all times the major part of activity in the liver was present in esterified form

Using doubly labeled chylomicrons BORGSTROM and JORDAN (1959) showed that chylomicron glycerides are taken up by the liver without previous hydrolysis Additional evidence for this can be obtained from consideration of the ratio labeled TGFA/labeled PLFA in the liver in the present experiment If the chylomicrons were hydrolyzed prior to their uptake by the liver this ratio would be expected to be the same as when FFA were injected In previous work (OLIVECRONA 1962) we found this ratio to be 3/2 at 5 min when 1 C^{14} palmitic acid was injected In the present experiment the ratio was approximately 20 1 at 10 min This is not consistent with the hypothesis that chylomicron glycerides are hydrolyzed prior to their uptake by the liver but rather supports the alternate hypothesis that they are taken up as glycerides

The carcass activity rose somewhat more slowly and reached its maximum later than the liver activity Also the carcass activity fell off at a slower rate When FFA was injected (OLIVECRONA 1962) both the liver and the carcass activities reached their maximal values within 10 min, and there was initially more activity going to the carcass and less to the liver than in the present experiment However the decline of activity in the tissues followed similar curves as those found in the present experiment After 2 hours the only major difference between the two series is that there was more activity in the adipose tissue in the present experiment With this exception it thus seems that although the mechanism of uptake of chylomicrons and FFA differ fatty acids injected in either form soon enter the same pools and share the same fate

The major difference between the metabolism of chylomicron fatty acids and free fatty acids thus seems to be that whereas the FFA are rapidly cleared from the blood stream and the cleared fatty acids almost immediately incorporated into tissue fatty acid esters the chylomicron fatty acids are cleared more slowly from the circulation and when cleared must first be hydrolyzed

from the glycerides before they can be further metabolized STEIN and SHAPIRO (1959 1960) have shown that lipids taken up by the liver cells are initially located on the actively metabolizing particles e g the mitochondrias and the microsomes They suggested that these lipids have a higher fractional turnover rate than the bulk of the liver fat A similar compartmentalization of the lipids probably occurs in most tissues When we inject a tracer dose of labeled FFA the label is accordingly rapidly taken up by the actively metabolizing particles and is soon oxidized or esterified The esters are then further metabolized either by hydrolysis and oxidation by mixing into the bulk of tissue fat, or — in the case of the liver — by transfer to the plasma. Thus there will be an initial rapid oxidation of the label which then levels off gradually as the fatty acids are mixed into successively bigger pools When we inject chylomicrons on the other hand the labeled fatty acids will enter the actively metabolizing pools more gradually because of the slower clearing from the blood and the hydrolysis necessary before the chylomicron fatty acids are available for further metabolism Thus the oxidation will initially proceed at a slower rate than for FFA but the rate of oxidation will level off later and more gradually We find those relations when we compare the curves for total recovery in the present experiment with those from the FFA experiment (OLIVECRONA 1962) The results of FREDRICKSON *et al* (1958) lend themselves to a similar interpretation

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From the Department of Physiology, Kungl. Veterinärhogskolan, Stockholm 51, Sweden

Effects of Chronic Central Cooling on Alimentation and Thermoregulation

By

B. ANDERSSON, C. C. GALE[1] and J. W. SUNDSTEN[2]

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Abstract

ANDERSSON, B., C. C. GALE and J. W. SUNDSTEN. *Effects of chronic central cooling on alimentation and thermoregulation.* Acta physiol. scand. 1962. *55.* 177—188. — Chronic local cooling in the forebrain of 4 unanesthetized goats was achieved by perfusion of cold water for 1—7 days through implanted silver thermodes. In 2 goats with thermodes located in the pre-optic region, chronic central cooling resulted in a marked inhibition of water consumption while causing little alteration of food intake. In a third goat with a thermode in the posterior pre-optic area and the rostral hypothalamus, central cooling inhibited water intake but also increased food intake slightly. In contrast, in a fourth goat bearing a thermode rostrally in the ventromedial hypothalamus, prolonged chilling resulted in a considerable increase in hay consumption while failing to inhibit drinking. These data provide additional evidence in support of the thermostatic theory of the regulation of food intake (BROBECK 1948) and of water intake (ANDERSSON and LARSSON 1961); further, they reveal a differential response in alimentation to cooling of separate areas of the forebrain. On central cooling, all thermode goats rapidly developed sustained hyperthermia without shivering, declining over 1—3 days upon cessation of central cooling. The sustained hyperthermia may be the result of peripheral vasoconstriction combined with increased metabolism of non-shivering origin. To explain the present results, there is no need to postulate the existence of central "cold receptors" or "hypothermia detectors" within the cooled part of the brain.

Experiments involving the production of acute thermal changes in the forebrain have been reported recently which provide support for the thermostatic theory of the regulation of food intake (BROBECK 1948) and which further

[1] Postdoctoral Research Fellow, Division of Neurological Diseases and Blindness, U. S. Public Health Service.

[2] Postdoctoral Fellow, Division of Biological and Medical Sciences, National Science Foundation.

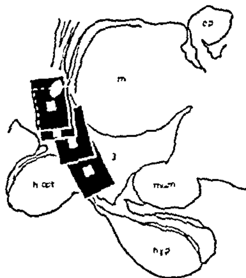


Fig. 1. Location of the thermodes in four goats subjected to local cooling of the forebrain.

Goats A and B. The thermode is located medially in the pre-optic region slightly more rostral and dorsal in A than in B.

Goat C. The thermode is situated medially in the posterior pre-optic and rostral hypothalamic region.

Goat D. The thermode is located medially in the rostral ventromedial hypothalamus.

ch. opt. = optic chiasm
ep. = epiphysis
hyp. = hypophysis
mam. = mammillary body
m. i. = massa intermedia
3 = the third ventricle

indicate the participation of a thermostatic mechanism in the regulation of water intake (ANDERSSON and LARSSON 1961). In order to investigate the thermostatic control of alimentation more thoroughly, it was thought important to study alimentary behaviour in goats subjected to more prolonged central thermal change. It also seemed of interest to study whether different locations of the thermodes within the pre-optic area and the rostral hypothalamus would influence the nature of the alimentary response to central cooling.

Thermoregulatory effects of acute cooling of the pre-optic area and the rostral hypothalamus have recently been studied in unanesthetized goats (ANDERSEN, ANDERSSON and GALE 1962). In the calm animal, accustomed to the experimental conditions, cooling of these parts of the forebrain produced shivering exclusively at environmental temperatures below 18° C, or during local stimulation of peripheral cold receptors. Since in these acute experiments general hyperthermia was seen to develop in the absence of shivering, it was of interest to study whether such non-shivering hyperthermia would persist during longer periods of central cooling.

Methods

Four horned goats were used in the present study. The animals had silver thermodes implanted medially: (1) in the pre-optic area (Goats A and B), (2) in the posterior pre-optic area and the rostral hypothalamus (Goat C), and (3) in the rostral ventromedial part of the hypothalamus (Goat D) (Fig. 1). Since the animals are still alive, the determination of the sites of the thermodes was made by X-ray studies of their heads. In 3 of the animals the degree of central cooling or warming was measured by needle applicators for temperature recording implanted with the thermosensitive tip 2 to 4 mm lateral to the surface of the thermodes. The construction of the thermodes and the methods of implantation and of central cooling and warming were previously described



Fig 2 *Experimental arrangement for chronic cooling of the forebrain by water perfusion of a permanently implanted thermode*

D = spring balanced drum
H = hay rack.
T = ice filled thermos (used optionally to induce deeper cooling)
Tu = rubber inflow and outflow tubings.
W = bucket for drinking water

(ANDERSSON and LARSSON 1961) Since however the periods of central cooling in the present series of experiments were prolonged up to a week, the technique of cooling had to be modified The animals were kept collared in metabolism cages and were not further restrained during the experimental periods During central cooling running tap water entered the thermode through a thin rubber tubing attached to the horn and was led out again through a similar rubber tubing connected to the cannula leading outwards from the thermode To speed up the perfusion rate a negative pressure was produced in the outlet tubing by water suction The rubber inlet and outlet tubings were suspended from a spring balanced drum 1 m above the heads of the animals The goats appeared to be completely indifferent to the presence of the perfusion apparatus This method of cooling caused a 3 to 4 C drop in the brain temperature 3 mm lateral to the thermode when perfusion was made in an environmental temperature of 18 to 21 C Somewhat deeper cooling (a 4 to 5 C drop in the brain temperature 3 mm lateral to the thermode) was obtained in other experiments. In these the tap water was led through a spiral copper tubing placed inside an ice filled thermos before it entered the rubber tubing leading to the thermode The experimental arrangements are seen in Fig 2

On several occasions the thermode was used also to produce local warming of the pre optic area for short periods of time in two of the animals. In these cases the temperature of the water running into the thermode cannula was kept at 46 C

Care of the animals

The technique of implantation of the thermodes made it possible to use the goats for these experiments for over a period of a year All 4 animals used in the present study are thus alive and in good condition They were routinely kept collared in metabolism

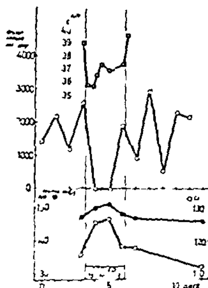


Fig 3 Total adipsia induced by 30 hour [illegible] cooling of the pre-optic region. At the onset of [illegible] water cooling in Goat B, the brain temperature dropped abruptly to 35 8 C and then rose slowly to 37 during the three-day cooling period Brain temperature was recorded 3 mm lateral to the thermode surface Drinking was totally blocked for two days, and when resumed on the third day it was insufficient to restore the water deficit incurred Plasma electrolytes rose during the period of adipsia.

cages and as stated above were not further restrained during the periods of central cooling All experiments were performed in an environmental temperature ranging from 18 to 21 C. The goats were fed daily 250 g of grain with 4 g of NaCl added and had free access to hay and water Daily records were kept of the amounts of hay and water consumed and of the amount and specific gravity of the urine excreted Two of the goats were lactating (Goats B and D) and were milked at regular times twice daily The milk production was recorded daily

Chemical methods

Urinary plasma and milk chloride was determined according to BRUN (1949) Sodium and potassium in these same body fluids were determined by use of an EEL flame photometer Blood sugar determinations were made according to the method of SOMOGYI (1945)

Results

I Results of acute warming in the pre optic region

Before the series of experiments involving chronic central cooling were started, Goat A was subjected to acute central warming by perfusion of warm water through its thermode This local warming of the pre optic region was observed to cause marked polydipsia on seven different occasions In addition to polypneic panting and peripheral vasodilatation local warming was thus seen to induce drinking after a latency of one to 3 min If the goat was allowed to drink large quantities of cold water during the first period of central warming it failed to drink again when central warming was performed a quarter of an hour later Further a preceding intramuscular injection of amphetamine (Phenopromin, ACO 1 5 mg kg body weight) completely inhibited the polydipsic effect of central warming

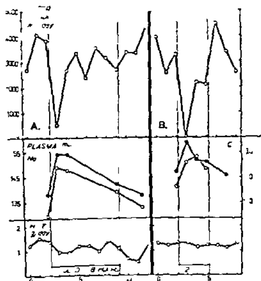


Fig 4 *Blockade of drinking prolonged by deeper cooling of the pre-optic region in Goat 4* Total adipsia lasted 7 hours following tap water perfusion (A) However adipsia was prolonged to 30 hours by perfusion with ice water (B) (this procedure reduced brain temperature about 1 C. further) Water intake remained low during the latter part of the cooling period, and then increased greatly on the first post-cooling day Plasma electrolytes rose steeply during cooling and returned to control levels after cooling

II *Effects of chronic central cooling on food and water intake*

A) *Thermode located in the pre optic region*

In 2 goats with thermodes implanted in the pre-optic region (Goats A and B Fig 1) continuous cooling with tap water for periods ranging from 1 to 7 days produced an immediate inhibition of water consumption while causing little or no reduction in the amount of hay eaten nor in the evident relish with which the morning grain ration was consumed Central cooling in every goat in this study was started simultaneously with the feeding of grain When these animals were not being centrally cooled they always drank the greatest part of their daily water intake within one to two hours after they had eaten their grain ration (which was usually consumed within 15 to 20 min) On the other hand when they were subjected to central cooling water intake was totally inhibited for periods ranging from seven to fifty hours This inhibition of the urge to drink was most pronounced in Goat B brain chilling by tap water perfusion on three separate occasions produced a complete inhibition of drinking lasting from 36 to 50 hours When this goat did break through this inhibition of thirst it drank relatively small amounts of water failing to make up its water deficit (Fig 3) This animal ate with good appetite during the cooling periods although on one occasion its consumption of hay declined on the second day

In Goat A drinking was not so severely inhibited as in Goat B and it began to drink small amounts of water after 7 hours of central cooling by tap water It was found possible however to prolong the period of total adipsia in this animal to over 30 hours by inducing a more profound degree of hypothermia in its pre-optic region (Fig 4) This was accomplished by perfusing its thermode

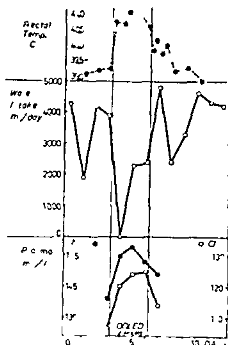


Fig 5 *Induction of core hyperthermia and inhibition of drinking by central cooling* In Goat C rectal temperature rose to 40 8 C within two hours of thermodal perfusion and persisted at this high level throughout central cooling Upon cessation of cooling rectal temperature fell slowly over a two-day period before reaching pre-cooling levels Water intake was totally blocked during the first 30 hours of cooling and remained low during the rest of the cooling period

with iced water instead of with tap water a procedure which apparently reduced the temperature in the cooled area an additional 1 C When this animal resumed drinking during this deeper central cooling it drank relatively small amounts of water failing to restore the water deficit it had occurred Upon stopping the central cooling a large increase in water consumption was observed on the first post cooling day

B) *Thermode located at the pre optic and rostral hypothalamic border*

In Goat C the thermode was located ventrocaudally to those in Goats A and B it was found to extend from the posterior part of the pre-optic region into the rostral portion of the hypothalamus (Fig 1 Goat C) Central cooling in this location elicited dual effects on alimentation causing both an inhibition of drinking and a stimulation of eating During a three day period of tap water cooling water intake was totally blocked for 30 hours and a condition of relative adipsia prevailed during the remainder of the cooling period (Fig 5) Although food intake was not markedly altered during this experiment when deeper cooling was later achieved (by iced water perfusion) an increased consumption of hay was observed

During the periods of absolute and relative inhibition of drinking in all three goats described above the plasma electrolyte concentration and the hematocrit rose sharply and usually did not decline to normal levels during the cooling despite the fact that all animals began to drink during the latter part of each

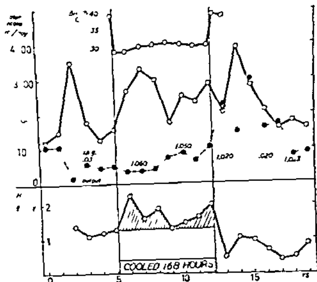


Fig 6 *Production of hyperphagia with no inhibition of drinking by cooling of the rostral ventromedial hypothalamus* In Goat D the consumption of hay increased an average of 40 during 7 days of central cooling The elaboration of urine of high specific gravity and low volume indicates that the supraoptico-hypophysial tract was not blocked by the central cooling Brain temperature was in this animal recorded 1 5 mm lateral to the surface of the thermode.

cooling period Upon cessation of central cooling these goats usually drank increased amounts of water which apparently was sufficient to restore their water deficits as indicated by the return of plasma electrolytes to normal concentrations (Fig 3 4 and 5)

C) *Thermode located in the rostral ventromedial hypothalamus*

In one goat bearing a thermode in the rostral ventromedial hypothalamus (Fig 1 Goat D) chronic brain chilling evoked different alimentary effects than in the 3 animals described above Central cooling on three separate occasions in Goat D caused it to eat increased amounts of hay while failing to inhibit its water intake For example during one period of tap water cooling lasting 7 days, hay consumption rose 40 % above control levels During the post cooling periods hay consumption was reduced below the pre-cooling control levels Central cooling (which in this goat must have involved the median eminence) did not inhibit the release of antidiuretic hormone since during cooling the urine output remained low while the specific gravity of the urine rose considerably On termination of cooling the diuresis of very large amounts of urine of low specific gravity was observed on two of the three occasions (Fig 6) Plasma electrolyte concentrations were little altered in this goat during cooling

III *Persistent hyperthermia produced by central cooling*

In view of the results of a recent study of the thermoregulatory effects of acute cooling in the pre-optic area and rostral hypothalamus of the goat (ANDERSEN *et al* 1962) is is not surprising to find in the present experiments that chronic central cooling never caused shivering but in every case evoked peripheral

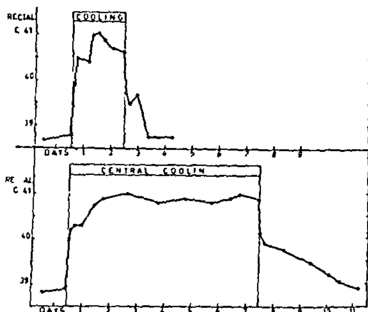


Fig 7 *Two examples of persistent hyperthermia evoked by central cooling in the same goat* In Goat D central cooling produced within two hours a rise in rectal temperature to 40 3 to 40 5 C a further rise occurred *8 to 12 hours later* On termination of a 3-day cooling period rectal temperature remained *elevated for one day* in contrast following a 7-day cooling period the core temperature *did not reach normal* level for 3 days

vasoconstriction It had been found in the earlier study that central cooling in calm goats accustomed to the experimental procedure induced shivering only in environmental temperatures below 18 C while peripheral vasoconstriction had occurred *in* environmental temperatures as high as 36 C In the present studies the rectal temperature of the animals started to rise very soon after the onset of central cooling and generally reached a temporary steady state within two to three hours A further rise usually occurred six to 12 hours later this was most evident in Goat D The rectal temperatures then remained relatively constant at a high level (40 5 to 41 5 C) during the entire period of central cooling even when prolonged for as long as 7 days On cessation of central cooling the rectal temperatures fell rapidly in association with the onset of marked peripheral vasodilatation and polypnea The rectal temperatures however did not immediately return to pre cooling levels but rather remained elevated for 1 to 3 days The longer the period of central cooling the longer was the duration of this post cooling elevation of the rectal temperature (Fig 5 and 7)

Although peripheral vasoconstriction (as indicated by a low ear surface temperature) was always present during the rising phase of rectal temperature it was less consistent when rectal temperature had reached the plateau level

Then the peripheral vasoconstriction was occasionally interrupted by brief periods of peripheral vasodilatation, a phenomenon which may explain observed small variations in the rectal temperature of the animals during central cooling

IV *Other effects of chronic central cooling*

During the periods of central cooling there was a moderate rise in blood glucose levels In animals with the thermode placed in the pre-optic region central cooling caused a 10 to 20 % increase in blood sugar level whereas cooling the rostral ventromedial hypothalamus raised the blood sugar 60 % (from 50 mg % to 80 mg %) Milk production was observed to remain at about the same level in Goat B (thermode in the pre-optic region) and to increase about 20 % in Goat D (thermode in the rostral ventromedial hypothalamus) during central cooling No consistent change in the pattern of urinary electrolytes could be related to cooling in any of the animals

Discussion

When trying to interpret the results of what may be thought physiological stimuli applied to various parts of the brain one is always faced with the question Is the effect due to an activation solely of neurons specifically sensitive to this particular stimulus or has the stimulus activated non specifically all surrounding nervous tissue? In the case of induced local hypothermia the problem becomes even more complicated for here at least four possibilities must be taken into consideration 1 The effects may be due to a specific inhibition of central warm receptors normally acting as a brake on various cold defense mechanisms 2 They may be due to a specific stimulation of central cold receptors or more accurately central hypothermia detectors 3 They may be due to a general non specific inactivation of all nervous tissue in the hypothermic area 4 Finally a fourth possibility must be taken into consideration a certain degree of hypothermia may alter the physical and chemical conditions of nervous tissue and thereby act as a non specific stimulus

Obviously the first possibility mentioned above must be at least part of the explanation of the effects obtained by chronic cooling of the pre-optic area and rostral hypothalamus The existence of central warm receptors within this region of the brain has been clearly demonstrated (MAGOUN et al 1938) Logically a local cooling of this region of the brain must inhibit the normal activity of such neurons and in this manner contribute to the development of general hyperthermia The thermoregulatory effects of acute cooling of the pre optic area in the goat (ANDERSSON et al 1962) could entirely be explained as due to such an inhibition of central warm receptors There was thus no need to postulate the existence of special "cold receptors (hypothermia detectors) within the cooled area of the brain The same applies to the present study Before discussing the alimentary effects on the basis of an inhibition of

central warm receptors, however one must consider the third possibility mentioned above i e that the central hypothermia might have inhibited all neuronal activity in the cooled region non specifically Even if such were the case the present study clearly demonstrates that the control of feeding and drinking is exerted by anatomically separate central mechanisms Thus cooling the rostral ventromedial hypothalamus caused an increased food intake but no simultaneous increase in water intake while cooling the pre optic region caused temporary adipsia without significantly altering the food intake of the goats. This observed increase in food intake could be therefore the result of a non specific inhibition of a ventromedial satiety center (BROBECK, TEPPERMAN and LONG 1943 Conversely the temporary adipsia could be caused by a non-specific inhibition of a slightly more dorsally located drinking center (ANDERSSON and McCANN 1955)

On the other hand certain features of the present study indicate that the local hypothermia has not been deep enough to inhibit non specifically all neuronal activity in the cooled region Thus for example the median eminence of Goat D (Fig 1 D) must have been cooled to the same or even greater extent as the ventromedial nucleus Still no sign of a diminished secretion of anti diuretic hormone was observed indicating that there was no cold blockade of the hypothalamico-neurohypophysial connections at any time during the period of central cooling Further evidence in support of this viewpoint is found in the observation that cooling in this same animal caused an increase in milk yield For if cooling had caused a non specific blockade in the median eminence milk production would be expected to decline secondary to deficiencies of those galactopoetic anterior pituitary hormones (such as ACTH and TSH) whose secretion is largely controlled by this region of the hypothalamus This study therefore indicates that central thermoreceptors may serve as an important link in the integration of alimentation and thermoregulation Thus the induction of local brain cooling was found to evoke vegetative changes which when brought into play would tend to correct a condition of general hypothermia the production of core hyperthermia a maintained or increased intake of food, and an inhibition of water intake (this latter action would prevent a further lowering of body temperature secondary to fluid ingestion) Conversely previous studies have shown that acute central warming induces vegetative changes tending to correct a condition of general hyperthermia vasodilatation and polypneic panting correlated with an increased water intake and a reduced food intake (ANDERSSON and LARSSON 1961)

According to the thermostatic theory of food intake hunger is reduced by a rise in temperature in the ventromedial nucleus of the hypothalamus (the satiety center) or in neurons located more rostral but which impinge on this nucleus (BROBECK 1960) In the present study however chronic cooling in the pre-optic region had little effect on food intake whereas hypothermia induced in the rostral ventromedial hypothalamus stimulated food consumption It is

possible that since cooling of the pre optic region caused a general hyperthermia the resultant perfusion of warm (40 5 to 41 5° C) blood through the brain served to raise the temperature in the satiety center and so to reduce the appetite to normal This would make necessary the postulation of warm receptors not only in the pre optic region but also in the rostral ventromedial hypothalamus Cooling performed directly in the satiety center would of course protect it from the warming effects of the core hyperthermia Spatial configuration tends to exclude the likelihood that hypothermia induced in the rostral ventromedial hypothalamus could extend sufficiently far laterally to influence the lateral hypothalamic feeding center (ANDERSSON and LARSSON 1961)

The area of the forebrain which upon cooling inhibited drinking most completely was localized to the pre optic region but in no case could total adipsia be prolonged beyond 50 hours That stimulation of the central osmoreceptors by a rise in plasma electrolyte concentration apparently served to break through this thermal blockade and to induce drinking suggests the existence of parallel mechanisms osmotic and thermostatic regulating water intake

The fact that local thermal change in the pre optic area affected the water intake of the animals to this great extent (local warming stimulating and local cooling inhibiting) is surprising in the view of earlier results of electrical stimulation in the same species A certain overlapping between the pre optic heat loss center and the hypothalamic drinking area was observed (ANDERSSON and McCANN 1955) but the most obvious polydipsic effect of electrical stimulation was found at a transverse level through the hypothalamus between the descending tract of the fornix and the mammillo-thalamic tract It has been recently observed however that extensive bilateral lesions in the pre optic region in the goat may cause permanent adipsia without blocking appetite in spite of the fact that the main part of the hypothalamus which on electrical stimulation normally causes drinking was untouched by the lesion (ANDERSSON and LARSSON 1962) The polydipsic effect of local warming of the pre optic area differed from that of electrical stimulation in two respects It was found to be inhibited by the previous drinking of cold water and also by the injection of amphetamine The continuation of drinking observed to follow the cessation of prolonged electrical stimulation in the hypothalamic drinking center was inhibited by these same factors but the immediate polydipsic effect of electrical stimulation was not so inhibited (ANDERSSON LARSSON and PERSSON 1960) It is difficult to interpret the importance of these observations before further information is obtained about the function of the pre optic area in the regulation of water intake Presently however the assumption appears justified that thermal and perhaps other factors influence the activities of the hypothalamic thirst center by way of the pre-optic area It is further possible that the activities of the hypothalamic thirst center must be propagated through the pre optic area in order to reach consciousness

In all goats in the present experiments central cooling quickly induced a high sustained core hyperthermia which persisted throughout the entire period of cooling. After cessation of cooling there initially occurred a rapid fall in rectal temperature to 0.5 to 1° C above normal levels. The further decline however, was slow and erratic. Rectal temperature thus did not reach pre-cooling levels until one to three days after stopping perfusion (Fig. 5 and 7). Since shivering was not present during any stage of the experiments the hyperthermia must have been due to the activation of other cold defense mechanisms. Peripheral vasoconstriction was always present during the rising phase of core hyperthermia, but later on was less consistent. It seems unlikely that peripheral vasoconstriction *per se* could have been sufficient to maintain the high body temperature for periods up to 7 days in an environmental temperature usually near 19° C. Although no measurements of oxygen consumption were made it seems justified to assume that an increase in metabolism of nonshivering origin had occurred during the periods of chronic central cooling in these animals. Evidence that an increased metabolism of non-shivering origin may be achieved during central cooling by way of the hypothalamico-hypophysial thyroid axis has recently been obtained in collateral studies in this laboratory. Thus, it was determined that local cooling of the pre-optic area in the goat rapidly leads to a marked increase in the protein bound iodine in the blood (ANDERSSON et al. 1962). Metabolic adjustments of this kind may explain the long delay before body temperature falls to normal levels after periods of chronic central cooling.

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From the Department of Physiology Karolinska Institutet Stockholm 60 Sweden

Effect of Ethanol on Oxygen Uptake and on Blood Glucose Concentration in Anesthetized Rabbits

By

E S PERMAN

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Abstract

PERMAN E S *Effect of ethanol on oxygen uptake and on blood glucose concentration in anesthetized rabbits* Acta physiol scand 1962 *55* 189—202 — In urethane anesthetized rabbits infusions of small doses of ethanol (0 2—0 4 g/kg) produced an immediate increase in oxygen uptake (10—15 per cent) There was also an increase in blood glucose The increase in oxygen uptake was apparently not primarily due to increased skeletal muscle activity or to in creased secretion from the adrenal medulla and it is suggested that ethanol or one of its metabolites has a direct stimulating effect on the metabolism The hyperglycemia was apparently due to increased secretion of adrenal medullary hormones — It is emphasized that these ethanol effects were obtained in a low dose range

It has recently been observed that ethanol in small doses (0 2 g/kg) increases oxygen uptake and blood glucose concentration in anesthetized rabbits (PERMAN 1961 a) These effects obtained in a dose range where other effects of ethanol are minimal have been studied further in the present investigation

The influence of ethanol on the oxygen uptake has been studied by several workers It has generally been concluded that in man ethanol has little or no effect on the oxygen uptake either in small or in large doses (HIGGINS 1917 GROLLMAN 1930 and others) In animals some investigators have noted increased oxygen uptake after ethanol doses ranging from 0 6 g/kg to 5 g/kg (WEISS and REISS 1923, LUNDSGAARD 1931 HEIM *et al* 1952 and others) whereas other workers have found no effect For review of the earlier literature on this subject see MITCHELL and CURZON (1940) — An increase in oxygen uptake after small ethanol doses (< 0 5 g/kg) in animals has apparently not been previously reported.

Earlier work has established that ethanol increases blood glucose under certain conditions. In most studies fairly large ethanol doses (> 3 g/kg) have been used, and it has been stressed that adequate stores of liver glycogen are required to obtain this effect (TENNENT 1941 and others). MATUNAGA (1942) showed that this type of ethanol hyperglycemia was reduced by section of the splanchnic nerves, and it has subsequently been confirmed that the rise in blood glucose after large ethanol doses is due to an increased adrenal medullary secretion (KLINGMAN and HAAG 1958, KLINGMAN, HAAG and BANE 1958, KLINGMAN, BANE and HAAG 1959, PERMAN 1961 b). KLINGMAN and her co-workers have also shown that ethanol intoxication is associated with increased activity in the sympathetic nervous system in general (KLINGMAN and GOODALL 1957, KLINGMAN *et al.* 1958). There is evidence that moderate ethanol doses (~ 1 g/kg) also increase the adrenal medullary secretion in man (PERMAN 1958, 1961 c, ABELIN, HERREN and BERLI 1958) and in cats (PERMAN 1960). — An increase in blood glucose after small ethanol doses (< 0.5 g/kg) has apparently not been previously reported.

In view of the well known effects of adrenaline on oxygen uptake and blood glucose, attention was directed towards evaluating the role of the adrenal medullary hormones in the metabolic actions of ethanol.

Methods

Albino rabbits weighing 2—5 kg were used. They were as a rule anesthetized with urethane 1.4 g/kg i.v. A few animals were anesthetized with pentobarbital, 40 mg/kg i.v. In some experiments the animals received 3 g glucose (10 ml 30 per cent glucose + 10 ml saline i.v.) at the same time as the anesthetic. This was done to ensure adequate liver glycogen stores.

The trachea was cannulated and connected, via membrane valves, to an oxygen-filled closed-circuit system for oxygen uptake determination according to standard technique (BENEDICT 1915). Measurements of the tracheal pressure variations during the experiments indicated that the resistance in the system was negligible. The spirometer had a capacity of 2 liters and a device which automatically refilled it with oxygen when it had been almost emptied, usually twice hourly during experiments, permitting continuous recording of the oxygen uptake curve. The initial oxygen uptake level, when the animal was in a steady state, was determined and expressed in ml/kg/min at 0° C, 760 mm Hg (dry). As the closed-circuit system had no device for water absorption, it was saturated with water vapor during experiments. Slight heating prevented water condensation in the expiratory valve. The spirometer temperature was checked at regular intervals. It varied little during experiments and was usually 26—28° C. The colonic temperature was continuously recorded with an electrical thermometer (ELECTROLAB TE 5, accuracy ± 0.1° C, with applicator RK 5). The body (colonic) temperature of the animals was maintained at 38—39° C during experiments.

The femoral vein was cannulated for fluid or drug administrations and the ipsilateral femoral artery for blood pressure recording. All animals were given heparin in amounts adequate for preventing coagulation (1 ml/kg of a 2 per cent solution). Blood glucose samples were obtained from a cannulated artery and determinations were carried out in duplicate using the method of HULTMAN (1959) which is specific for aldo-

Table 1 Effect of an intravenous infusion of 0.2 g/kg ethanol on oxygen uptake and rectal temperature in 6 urethane anesthetized (1.4 g/kg) male albino rabbits The animals had received 3 g glucose 1—4 hours before ethanol

Exp no.	Weight kg	Initial oxygen uptake ml/kg/min	Rectal temp. before ethanol C	Ethanol infusion		Effect on oxygen uptake		Constant change in rectal temp C
				Infusion time min	Ethanol conc. g/ml	Largest increase (during 10 min period) per cent	Approx. duration of increase min	
1	2.7	8.2	38.5	7	0.03	12	60	+0.4
2	2.8	9.0	38.6	7	0.03	10	40	—0.1
3	3.0	7.7	38.5	8	0.03	12	60	—0.3
4	3.0	7.8	38.6	8	0.03	17	60	+0.1
5	3.0	8.0	38.5	8	0.03	11	60	+0.5
6	4.6	6.6	38.6	6	0.16	11	60	—0.2
	Mean	7.9				12		

Animal cooled during experiment.

saccharides Blood ethanol was determined in duplicate samples with the WIDMARK (1932) method

Ethanol solutions were made up from absolute ethanol (99.5 per cent by volume) and saline (0.9 per cent) The highest ethanol concentration used in any of the present experiments was 0.16 g/ml Pure saline or distilled water was used in control infusions. Solutions of glucose dissolved in saline were administered in a similar fashion The intravenous infusions were made with a motor-driven infusion apparatus set at a constant rate of 1.0 ml/min All solutions were administered at room temperature Adrenaline solutions were made up from a standard solution (100 μg/ml) which was diluted to the required concentration with saline immediately before administration In a few experiments the animals were curarized An initial dose of 1.5 mg d-tubocurarine was given and small additional doses if required These animals were artificially ventilated with a STARLING pump connected to the closed-circuit system so as to permit recording of oxygen uptake Ganglionic blockade was obtained by an intravenous injection of 10 mg/kg hexamethonium bromide when required At the beginning of some experiments the adrenals were ligated via a midline incision In other experiments the splanchnic nerves were sectioned bilaterally as close to the adrenal glands as possible

Results

1 Oxygen uptake and body temperature

a) Ethanol infusion experiments

In 6 urethane anesthetized rabbits a small ethanol dose (0.2 g/kg) was infused and its effects on oxygen uptake and body temperature are shown in

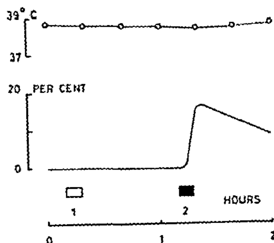


Fig 1 Rabbit ♀ 3 0 kg Urethane anesthesia 1 4 g/kg 3 g glucose given 4 hours before experiment Initial oxygen uptake level 7 9 ml/kg min. From above downwards Rectal temperature in °C. Change in oxygen uptake in per cent of initial level Time scale in hours
1 Control infusion of saline i v
2 Ethanol infusion 0 2 g/kg i v

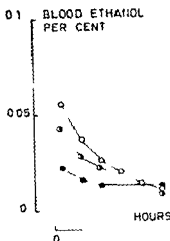


Fig 2 Blood ethanol curves from 3 rabbits anesthetized with urethane 1 4 g/kg 0 2 g/kg ethanol was infused at a constant rate during 7—9 min and beginning of time scale coincides with end of infusion

Table I The average increase in oxygen uptake was 12 per cent in these experiments The oxygen uptake level prior to ethanol administration was very stable The low uptake value in exp no 6 is probably related to the comparatively high body weight of that animal — The rectal temperature increased gradually after ethanol in two experiments Infusions of saline distilled water or glucose (0 1 and 0 3 g/kg) had no appreciable effect on oxygen uptake or on rectal temperature Fig 1 is a diagram of one of the ethanol experiments (no 4)

The oxygen uptake curve showed clearly in all experiments that the oxygen uptake increased during the ethanol infusion when an amount corresponding to about 0 1 g/kg had been infused and that it rapidly reached its maximum level The oxygen uptake remained elevated for about 40—60 min in these experiments After this time it was usually back at its "pre ethanol" level

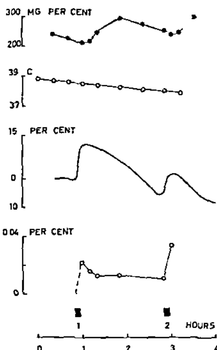


Fig 3 Rabbit ♂ 4 6 kg Urethane anesthesia 1 4 g/kg 3 g glucose given 2 hours before experiment Initial oxygen uptake level 6 6 ml/kg/min From above downwards Blood glucose concentration in mg per cent Rectal temperature in C Change in oxygen uptake in per cent of initial level Blood ethanol concentration in per cent Time scale in hours
1 Ethanol infusion 0 2 g/kg i v
2 — » —

Ethanol infusions of 0 01 g/kg and 0 05 g/kg failed to increase oxygen uptake In other experiments the effects of larger ethanol doses (0 2—5 0 g/kg) were studied It was found that as a rule the increase in oxygen uptake caused by 0 2— 0 4 g/kg ethanol was not further augmented by additional amounts of ethanol When larger ethanol doses were infused there was during the latter part of the infusion a decrease in oxygen uptake concomitant with the depressive effects on circulation and respiration After the increase in oxygen uptake produced by ethanol had subsided a second ethanol infusion had a similar effect usually however of smaller magnitude (Fig 3)

The blood ethanol concentration was followed in 3 experiments after the infusion of 0 2 g/kg ethanol i v The curves are shown in Fig 2 Within the first 5 min after the end of the infusion blood ethanol concentrations of 0 022—0 056 per cent were obtained The subsequent fall in concentration was rapid and after one hour the mean value was 0 01 per cent It should be noted that in the experiment where the peak blood ethanol concentration was only 0 022 per cent the oxygen uptake increased 12 per cent (Fig 3)

In all the experiments described above the ethanol effect on oxygen uptake was obtained in male rabbits which had been pretreated with glucose Subsequent experiments on female rabbits not pretreated with glucose gave similar

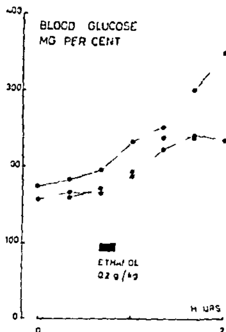


Fig 4 Effect of an intravenous infusion of 0 2 g/kg ethanol on blood glucose in 3 urethane-anesthetized (1 4 g kg) rabbits

results Small additional doses of urethane given during the experiments to maintain adequate anesthesia did not affect oxygen uptake Oxygen uptake increased after ethanol also when pentobarbital was used as anesthetic

It was noted in some experiments that there was no or only a small (< 5 per cent) increase in oxygen uptake following the infusion of 0 2 g/kg ethanol In a few of these experiments the animals responded with the usual increase when a higher dose (0 4 g/kg) was given In other experiments the lack of effect was probably related to the fact that the animals were in hyperthermia or in hypothermia with a correspondingly affected initial oxygen uptake. In a few experiments however the reason for the lack of effect was unclear

b) Ethanol injection experiments

When ethanol was injected within 10 sec instead of being administered as an infusion the threshold dose was lower The injection of 0 08 g/kg ethanol caused a short lasting (10—15 min) increase in oxygen uptake of about 10 per cent and 0 16 g/kg caused a similar increase of longer duration Control injections of saline were without effect

2 Respiration and blood pressure

The rate and depth of the respiration were unaffected by the infusion of 0 2 g/kg ethanol as indicated by the oxygen uptake curve and by direct observation In no experiment was the ethanol infusion followed by any immediate clearcut increase in respiration

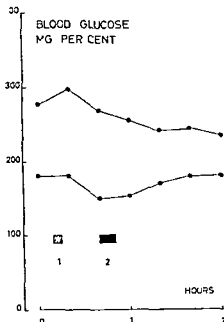


Fig 5 Effect of an intravenous infusion of 0.2 g/kg ethanol on blood glucose in 2 urethane anesthetized (1 4 g/kg) rabbits after hexa methonium
1 Hexamethonium bromide 10 mg/kg i v
2 Ethanol infus on 0 2 g/kg v

The blood pressure which was continuously recorded in all experiments was not affected by 0 2 g/kg ethanol whereas 0 4 g/kg sometimes caused a moderate (10—20 mm Hg) short lasting rise

No evidence was obtained that ethanol in the dose range 0 1—0 3 g/kg when injected or infused had other than minor and short lasting effects on respiration or blood pressure

3 Blood glucose concentration

In urethane anesthetized rabbits blood glucose increased after infusion of 0 2 g/kg ethanol Blood glucose curves from 3 rabbits given this dose are shown in Fig 4 The initial level averaged 180 mg per cent The rise after ethanol was gradual and as a rule the peak blood glucose value was attained within one hour after the end of the infusion The average increase was 70 mg per cent in these experiments Infusing saline in comparable amounts had no similar effect but a slight increase was noted

In similar ethanol experiments on animals pretreated with glucose (Table II) the initial blood glucose level was higher (about 300 mg per cent) Also in these experiments blood glucose increased

The results were similar when rabbits of both sexes were used as well as in experiments where pentobarbital was used as anesthetic

Table II. Effect of an intravenous infusion of 0.2 g/kg ethanol on blood glucose in 3 urethane-anesthetized (1.4 g/kg) male albino rabbits pretreated with 3 g glucose 1—4 hours before ethanol

Exp. no.	Initial blood glucose level mg per cent	Highest obtained blood glucose value within 1 hour after infusion of ethanol mg per cent	Increase in blood glucose mg per cent
1	3.0	488	138
2	146	320	174
3	384	462	78
Mean	~ 2.0	~ 4 0	~ 130

4. Curarization and artificial respiration

Table III gives data from two experiments on urethane anesthetized and curarized rabbits maintained on artificial respiration during the experimental period. The oxygen uptake increased after infusion of 0.2 g/kg ethanol, and the effect was similar to that obtained in spontaneously breathing animals (see Table I). The animal in exp. no. 1 was pretreated with glucose and blood glucose was followed. It increased from 234 mg per cent to 354 mg per cent within one hour after ethanol. Small maintenance doses of d-tubocurarine given during the experiments did not affect the oxygen uptake.

Table III. Effect of an intravenous infusion of 0.2 g/kg ethanol on oxygen uptake in 2 urethane-anesthetized (1.4 g/kg) male albino rabbits curarized and maintained on artificial respiration

Exp. no.	Weight	Initial oxygen uptake ml/kg/min	Rectal temp. before ethanol °C	Ethanol infusion		Effect on oxygen uptake	
				Infusion time min	Ethanol conc. g/ml	Largest increase (during 10 min period) per cent	Approx. duration of increase min
1	2.8	9.8	38.0	7	0.08	10	60
2	2.2	8.9	38.0	6	0.08	14	> 40

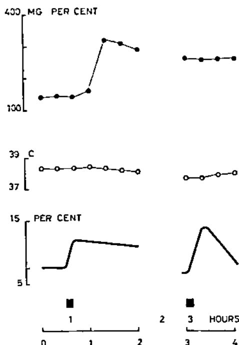


Fig 6 Rabbit ♂ 3 0 kg Urethane anesthes a 1 4 g/kg 3 g glucose given 3 hours before experiment Initial oxygen uptake level 8 1 ml/kg/min
From above downwards Blood glucose concentration in mg per cent Rectal temperature in C Change in oxygen uptake in per cent of initial level Time scale in hours
1 Ethanol infusion 0 2 g/kg i v
2 Hexamethonium bromide 10 mg/kg i v
3 Ethanol infusion 0 2 g/kg i v

5 Ganglionic blockade with hexamethonium

Ganglionic blockade with 10 mg/kg hexamethonium bromide produced a marked blood pressure fall (to about 40 mm Hg) after which the pressure stabilized itself at 60—70 mm Hg It was observed that the oxygen uptake also fell to a lower level

Ethanol infused within one hour after hexamethonium administration increased oxygen uptake The range of increase was 10—17 per cent in 3 animals when a dose of 0 2 g/kg was infused However the blood glucose did not increase after ethanol in similar experiments Fig 5 shows the lack of effect of ethanol on blood glucose in two animals given hexamethonium Fig 6 shows the effect of an infusion of 0 2 g/kg ethanol on oxygen uptake and on blood glucose before and after ganglionic blockade in another rabbit which had been pretreated with glucose

6 Ligation and denervation of the adrenals

In animals where the influence of the adrenal medullary secretion was abolished by acute ligation of the glands or by sectioning the splanchnic nerves ethanol (0 2 g/kg) still caused an increase in oxygen uptake The effect was of smaller magnitude and had a shorter duration than in untreated animals This was probably related to an impairment of the general con

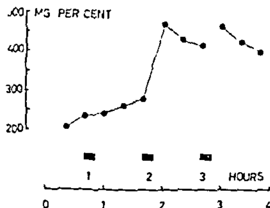


Fig 7 Effect of 3 different adrenaline doses on blood glucose in a urethane anesthetized (1 4 g kg) rabbit given 3 g glucose 4 hours before experiment
1 0 35 μg kg adrenaline i v Infusion rate 0 035 μg kg/min
2 3 5 μg kg adrenaline i v Infusion rate 0 35 μg kg min
3 35 μg kg adrenaline i v Infusion rate 3 5 μg kg min.

dition associated with the operative procedure A fall in blood pressure and body temperature and a reduction of oxygen uptake was always seen after acute ligation or denervation of the adrenals

7 Administration of adrenaline

The influence of infused adrenaline on oxygen uptake and on blood glucose was investigated Ten minute intravenous infusions of adrenaline at rates of 0 035 0 35 and 3 5 μg/kg/min were made No immediate clearcut increase in oxygen uptake was noted concomitantly with any of the adrenaline in usions whereas the blood glucose increased immediately after the two higher doses (Fig 7) The blood pressure was slightly increased by the medium dose and markedly increased (+ 40 mm Hg) by the large dose A similar lack of effect on oxygen uptake with these adrenaline doses was noted in experiments on curarized rabbits maintained on artificial respiration Adrenaline injections in the dose range 0 2—1 1 μg/kg did not affect oxygen uptake

Thus none of the adrenaline doses employed in the present experiments caused any increase in oxygen uptake comparable to that which could subsequently in the same animal be obtained with ethanol However moderate doses of adrenaline increased blood glucose and this was independent of whether or not the animal had received pretreatment with glucose

Discussion

The results show that ethanol in small doses (0 1—0 4 g/kg) increases oxygen uptake and blood glucose concentration in anesthetized rabbits Urethane was used as anesthetic in most experiments but similar results were obtained with Nembutal The anesthesia was as a rule light and although its influence

on the present results is not known it seems reasonable to assume that anesthesia decreases the sensitivity of the organism to small ethanol doses There was no evidence for a sex difference with regard to the effect of ethanol on oxygen uptake or blood glucose A sex difference in tolerance to higher ethanol doses under certain conditions has been noted (WALLGREN 1959)

The animals were in good condition during the experiments and the pre ethanol oxygen uptake level (6 6—9 0 ml/kg/min) was stable and not affected by infusion of saline, distilled water or glucose solutions Slightly higher resting oxygen uptake levels (7 7—11 8 ml/kg/min) were obtained with similar technique in un anesthetized rabbits by LUNDHOLM (1949 pp 104—105) It has been shown in rabbits (TANGL and VERZAR 1918) and guinea pigs (LUNDHOLM 1949 p 53) that the oxygen uptake is moderately decreased during urethane anesthesia

No evidence of increased muscle activity was obtained and no increase in respiration which could account for the increase in oxygen uptake was noted A similar increase in oxygen uptake after ethanol was also seen in curarized animals which seems to exclude the possibility that the increased oxygen uptake is due to increased activity of skeletal muscle Earlier work has shown that ethanol can increase the respiration moderately (HIGGINS 1917 and others), apparently via the chemoreceptors (GERNANDT 1943 LANDGREN LILJESTRAND and ZOTTERMAN 1953) HEIM *et al* (1952) studying the effect of monosaccharides on the acute tolerance to infusions of ethanol of fasted chloralose anesthetized cats noted a transitory increase in oxygen uptake during the ethanol infusion which they ascribed to a concomitant increase in respiration

Several findings indicate that the increase in oxygen uptake after ethanol is not due to an increased secretion from the adrenal medulla If increased adrenal medullary secretion were responsible for the increased oxygen uptake after ethanol one would expect a similar increase in oxygen uptake when adrenaline was infused as adrenaline is the rabbit s chief adrenal medullary hormone (HOKFELT and MCLEAN 1950) However it was not possible to produce a similar increase in oxygen uptake with short term infusions of adrenaline even when doses were used which caused hyperglycemia and pressor effects This is in agreement with the results of LUNDHOLM (1949 p 117) who obtained no distinct increase in oxygen uptake during the first 10—15 min of adrenaline infusions (0 5—2 µg/kg min) in un anesthetized rabbits and with those of KLEIN and WEISS (1928) who found no stimulation of oxygen uptake after adrenaline in urethane anesthetized rabbits Ganglionic blockade did not abolish the increase in oxygen uptake after ethanol Finally experiments on animals with ligated or denervated adrenals furnished no evidence that ethanol increases oxygen uptake via the adrenal medulla The increased adrenal medullary secretion after ethanol administration in the present experiments indicated by the increase in blood glucose may however have contributed secondarily to the increase in oxygen uptake

It is tempting to speculate on how ethanol might increase oxygen uptake It is known from earlier work that ethanol increases oxygen uptake of various tissues *in vitro* (ROBERTSON and STEWART 1932 GHOSH and QUASTEL 1954 WALLGREN and KULONEN 1960, and others) and a similar *in vivo* effect seems compatible with the present results The increase might be caused by acetaldehyde formed during ethanol metabolism TRUITT BELL and KRANTZ (1956) using brain tissue have shown that moderate acetaldehyde concentrations have an uncoupling effect on oxidative phosphorylation which increases oxygen uptake Acetaldehyde in small doses (0 04 g/kg), when infused causes a transitory increase in oxygen uptake similar to that caused by 0 2 g/kg ethanol (PERMAN 1962) The rate of acetaldehyde formation from ethanol *in vivo* is apparently maximal at low blood ethanol concentrations (< 0 02 per cent) due to the high affinity between ethanol and alcohol dehydrogenase (BONNICHSEN and THEORELL 1951) An effect via acetaldehyde could explain why the increase in oxygen uptake caused by ethanol occurs at these low blood ethanol levels It is also possible, however that the effect of ethanol on oxygen uptake is related to its depolarizing action on membranes (GALLEGO 1948)

The initial blood glucose values were high (about 180 mg per cent) in most experiments This seems to be due to a high resting adrenaline output from the adrenal medulla probably due to the anesthesia and preparation, since the blood glucose decreased after hexamethonium administration The increase in blood glucose after ethanol in the present experiments seems to have been caused by increased adrenal medullary secretion since it was reduced or abolished by hexamethonium These findings are in agreement with those obtained in the dog by KLINGMAN and co-workers (KLINGMAN *et al* 1959) although smaller ethanol doses were employed in the present study This may be related to the fact that the minimal hyperglycemic adrenaline dose is low in the rabbit in comparison with other species (ELLIS 1956)

Further studies of these ethanol effects might provide a new background for the controversial stimulating effect of small ethanol doses

This work has been supported by grants from the Alcohol Research Committee of the Swedish Medical Research Council and from Stiftelsen Lars Hiertas minne Blood ethanol determinations were carried out by Mrs A HULTÉN at the Department of Alcohol Research, Karolinska Institutet to whom the author expresses his thanks

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From the Department of Physiology Karolinska Institutet Stockholm 60 Sweden

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E S PERMAN

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PERMAN E S *Effect of methanol n propanol and acetaldehyde on oxygen uptake and on blood glucose concentration in anesthetized rabbits* Acta physiol scand 1962 *55* 203—206 — Increases in oxygen uptake and in blood glucose concentration after ethanol administration have recently been demonstrated in urethane anesthetized rabbits The present experiments were performed to determine whether substances related to ethanol produce similar effects The substances were infused intravenously — Methanol and n propanol did not affect the oxygen uptake in doses comparable to the effective ethanol doses This suggests that the effect of ethanol on oxygen uptake is not common to lower primary alcohols Acetaldehyde, the chief primary ethanol metabolite increased oxygen uptake in a smaller dose It is tentatively suggested that acetaldehyde is involved in the production of increased oxygen uptake noted after ethanol — Blood glucose increased only after methanol administration The increase was not abolished by ganglionic blockade suggesting that methanol and ethanol raise the blood glucose level via different mechanisms

It has recently been found (PERMAN 1961 1962) that ethanol in small doses (0 2 g/kg) produces an immediate moderate increase in oxygen uptake and a gradual rise in blood glucose in urethane anesthetized rabbits It was shown that the increase in oxygen uptake was not due to activity of skeletal muscle but probably to a direct ethanol effect on cellular respiration A similar stimulating effect of ethanol on the oxygen uptake of various tissues

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Acta physiol. scand 1962 55 203—206

From the Department of Physiology Karolinska Institutet, Stockholm 60 Sweden

Effect of Methanol, N-Propanol and Acetaldehyde on Oxygen Uptake and on Blood Glucose Concentration in Anesthetized Rabbits

By

E S PERMAN

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Abstract

PERMAN E S *Effect of methanol n propanol and acetaldehyde on oxygen uptake and on blood glucose concentration in anesthetized rabbits* Acta physiol scand 1962 *55* 203—206 — Increases in oxygen uptake and in blood glucose concentration after ethanol administration have recently been demonstrated in urethane anesthetized rabbits The present experiments were performed to determine whether substances related to ethanol produce similar effects. The substances were infused intravenously — Methanol and n propanol did not affect the oxygen uptake in doses comparable to the effective ethanol doses This suggests that the effect of ethanol on oxygen uptake is not common to lower primary alcohols Acetaldehyde, the chief primary ethanol metabolite increased oxygen uptake in a smaller dose It is tentatively suggested that acetaldehyde is involved in the production of increased oxygen uptake noted after ethanol. — Blood glucose increased only after methanol administration The increase was not abolished by ganglionic blockade suggesting that methanol and ethanol raise the blood glucose level via different mechanisms.

It has recently been found (PERMAN 1961 1962) that ethanol in small doses (0 2 g/kg) produces an immediate moderate increase in oxygen uptake and a gradual rise in blood glucose in urethane anesthetized rabbits It was shown that the increase in oxygen uptake was not due to activity of skeletal muscle but probably to a direct ethanol effect on cellular respiration A similar stimulating effect of ethanol on the oxygen uptake of various tissues

Table I. Effect of methanol, ethanol, n-propanol and acetaldehyde on the oxygen uptake of urethane anesthetized rabbits

Substance	No. of exp	Dose		Infusion time min	Effect on oxygen uptake	
		g/kg	mmoles/kg		Largest increase (during 10 min period) per cent	Approximate duration of effect after end of infusion min
Methanol CH_3OH	3	0.14	4.4	6–8	0	–
Ethanol[1] CH_3CH_2OH	6	0.20	4.4	6–10	10–15	40–60
n-Propanol $CH_3CH_2CH_2OH$	3	0.26	4.4	6–9	0	–
Acetaldehyde CH_3CHO	3	0.04	0.87	6–9	10–14	5–10

[1] Data from PERMAN (1962).

in vitro has been observed under certain conditions (ROBERTSON and STEWART 1932, WALLGREN and KULONEN 1960 and others). The rise in blood glucose was apparently due to increased secretion from the adrenal medulla. Increased adreno-medullary secretion after ethanol administration has been reported earlier (KLINGMAN and GOODALL 1957, PERMAN 1958 and others).

It was considered of interest to see if compounds closely related to ethanol produce similar effects. Results of experiments on methanol, n-propanol and acetaldehyde are given in the present paper.

Methods

The methods used in the present experiments have been described in an earlier study on ethanol (PERMAN 1962). The main features were as follows. Albino rabbits anesthetized with urethane (1.4 g/kg) were used. Oxygen uptake was determined according to conventional BENEDICT technique with the animal connected to the closed-circuit system via a tracheal cannula. Solutions for infusion were made with saline (0.9 per cent). The final concentration of methanol and n-propanol was 1.74 mmoles/ml. The acetaldehyde concentration used was 0.35 mmoles/ml. The solutions were administered intravenously at a slow, constant rate (1.0 ml/min). The blood pressure was continuously recorded from the femoral artery after heparinization. Blood glucose was determined with the aldosaccharide specific method of HULTMAN (1959). The body (colonic) temperature was maintained at 38—39° C.

Results

The results of the oxygen uptake experiments are summarized in Table I. Oxygen uptake was unaffected by methanol and n-propanol in doses comparable (on a molar basis) to the ethanol dose which regularly produced increased oxygen uptake (4.4 mmoles/kg). When acetaldehyde was administered

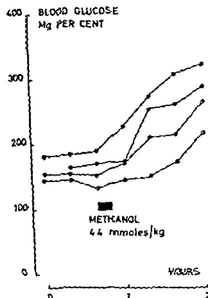


Fig 1 Effect of a methanol infusion (4 4 mmoles/kg) on blood glucose of urethane anesthetized rabbits.

●—● untreated animals.

◐—◐ 10 mg/kg hexamethonium bromide given i. v. between first and second blood glucose sample.

in a dose corresponding to one fifth of the effective ethanol dose there was an immediate moderate increase in oxygen uptake which however lasted only a short time after the infusion was discontinued This acetaldehyde effect was also seen in curarized artificially ventilated animals Blood glucose was not consistently affected by the n propanol and acetaldehyde infusions Methanol (Fig 1) produced a blood glucose rise similar to that seen after administration of ethanol The rise was however not abolished by ganglionic blockade with hexamethonium bromide (10 mg/kg i v) The blood pressure and general condition of the animals were mainly unaffected during the experiments during infusions of n propanol there was sometimes a slight blood pressure fall

Discussion

The present results show that the increase in oxygen uptake produced by ethanol is not a non specific effect common to lower primary alcohols The observation that the oxygen uptake is similarly increased during infusions of small acetaldehyde doses suggests that ethanol increases the oxygen uptake *in vivo* via acetaldehyde its chief primary breakdown product (*cf* JACOBSEN 1952) TRUITT BELL and KRANTZ (1956) found a limited degree of uncoupling of oxidative phosphorylation leading to increased oxygen uptake in brain tissue *in vitro* in the presence of acetaldehyde in moderate concentrations but no similar effect of ethanol and its homologues up to n butanol An *in vivo* effect via acetaldehyde could explain why the effect occurs after these small ethanol

doses, as the high affinity between ethanol and alcohol dehydrogenase (BONNICHSEN and THEORELL 1951) apparently leads to a maximal rate of acetaldehyde formation at low ethanol concentrations (0 02 per cent) The rapid metabolism of acetaldehyde in the organism could account for the short duration of the effect of infused acetaldehyde on oxygen uptake

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WALLGREN H and E KULONEN Effect of ethanol on respiration of rat brain cortex slices *Biochem J* 1960 *75* 150—158

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From the Department of Physiology Karolinska Institutet, Stockholm 60 Sweden

Increase in Oxygen Uptake After Small Ethanol Doses in Man

By

F S PERMAN

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Abstract

PERMAN E. S *Increase in oxygen uptake after small ethanol doses in man* Acta physiol. scand. 1962 55 207—209 — It has generally been concluded from earlier work that ethanol has little or no effect on oxygen uptake in man. The present experiments were made on 7 healthy young men Oxygen uptake was determined before and after intake of a small ethanol dose (0 3 g/kg) and in control experiments without ethanol The oxygen uptake was moderately increased 20—50 min after intake of ethanol No concomitant increases in respiratory rate, heart rate or general motor activity were seen.

It is firmly established by earlier work that ethanol is metabolized in the organism and its nutritional value is well known. Its influence on oxygen uptake in man under various conditions has been studied by several workers and although a slight increase in oxygen uptake has sometimes been noted (MONALDI 1931 and others) most workers have found no change (HIGGINS 1917 GROLLMAN 1930 MEYER 1931 and others) It has therefore generally been concluded (for review see MITCHELL and CURZON 1940) that ethanol has little or no effect on oxygen uptake either in low or in high doses

It was recently noted (PERMAN 1961 1962) that an intravenous infusion of a small ethanol dose (0 2 g/kg) produces an immediate moderate (10—15 per cent) increase in the oxygen uptake of anesthetized rabbits, as measured with the BENEDICT technique Further it was shown that this increase was not due to activity of skeletal muscle but probably to a direct ethanol effect on cellular respiration The present paper reports experiments in which the effect of small ethanol doses on the oxygen uptake of human subjects was re investigated

Table I Oxygen uptake in ethanol and control experiments on 7 human subjects

Subject no	Control experiment Oxygen uptake			Ethanol experiment Oxygen uptake			
	Initial ml/min	Change in per cent		Ethanol dose g/kg	Before ethanol ml/min	Change in per cent	
		20—50 min	70—100 min			20—50 min	70—100 min
1	[illegible]06	—6		0 34	2[illegible]6	+4	
2	282	0		0 27	2[illegible]9	+7	
3	2[illegible]	—4		0 31	318	+7	
4	272	—3		0 30	2[illegible]0	+7	
5	284	0	—4	0.27	2[illegible]6	+6	—1
6	249	—3	—7	0 29	233	+4	+1
7	314	—8	—9	0 27	30[illegible]	+4	—1
	Mean	—2 3				+5 [illegible]	

Methods

Seven healthy young men (age 20—24 years) all with modest ethanol habits served as test subjects They had previous experience with oxygen uptake determinations The experiments were carried out in the afternoon without previous fasting thus not under basal conditions but the subjects had to rest for 30 min before determination of the initial oxygen uptake level. Conventional KROGH technique was used for the oxygen uptake determination and each value is the mean of two consecutive 10 min determinations After determining the initial oxygen uptake level 150 ml chilled fruit juice to which 30 ml of a 71 per cent (w/v) ethanol solution had been added was given to the test subject to drink within 10 min This amount of ethanol corresponded to a dose of 0 3 g/kg and produced as expected no gross signs of intoxication Twenty to fifty and in a few experiments 70—100 min after ethanol intake the oxygen uptake was again determined and its percentage change from the initial value was computed Control experiments with fruit juice were carried out one day before or after the ethanol experiments The subjects were recumbent during the whole experiment.

Results

The results are shown in Table I The initial oxygen uptake levels were reasonably similar in the two groups In the ethanol experiments there was a small increase (average 6 per cent) in oxygen uptake 20—50 min after intake which was not seen in the control experiments The oxygen uptake was back to its pre-ethanol level 70—100 min after ethanol intake In several experiments the respiratory rate and the heart rate were determined concomitantly with the oxygen uptake and were found to be unchanged The blood ethanol concentration was determined in some experiments according to WIDMARK (1932) and values ranging from 0 018 to 0 034 per cent were obtained 50—55 min after ethanol intake

Discussion

No evidence was obtained that the increase in oxygen uptake after ethanol was due to increased muscular activity Because of its modest magnitude it may have been overlooked by earlier workers The increase may be of the same nature as that seen in anesthetized rabbits and could be related to the ability of ethanol to increase the oxygen uptake of various tissues *in vitro* under certain conditions (WALLGREN and KULONEN 1960 and others)

This work was supported by a grant from the Alcohol Research Committee of the Swedish Medical Research Council Blood ethanol determinations were carried out by Mrs A. HULTÉN at the Department of Alcohol Research Karolinska Institutet to whom the author expresses his thanks

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Acta physiol. scand. 1962. 55. 210—224

From the Laboratory of Clinical Neurophysiology, Department of Neurology, University of Lund, Sweden, and the Institute of Physiology, University of Cologne, Germany

Normal and Epileptic EEG Patterns Related to Cortical Oxygen Tension in the Cat

By

DAVID H. INGVAR, DIETRICH W. LÜBBERS and BO K. SIESJÖ

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Abstract

INGVAR, D. H., D. W. LÜBBERS and B. K. SIESJÖ. *Normal and epileptic EEG patterns related to cortical oxygen tension in the cat.* Acta physiol. scand. 1962. 55. 210—224. — The oxygen tension of the cerebral cortex was measured in relative terms with a polarographic technique (membrane covered platinum electrode of the CLARK type, LÜBBERS 1960) in lightly or non anaesthetized cat preparations of the *encéphale* or *cerveau isolé* type. In steady states with controlled systemic circulation and respiration the cortical pO_2 measured was found to be stable. Only small changes in cortical pO_2 were seen during periods of slowly changing EEG patterns. Acute EEG changes, on the other hand, induced by electrical stimulation or chemically, were accompanied by regular reproducible changes in cortical pO_2 indicating — under these conditions — a correlation between EEG pattern and cortical oxygen consumption. The arousal reaction of the EEG elicited by electrical stimulation of the meso-diencephalic brain stem was studied especially. It was accompanied by a biphasic change in cortical pO_2 with a small initial decrease and a more constant subsequent large increase proportional to stimulus strength and of duration about equal to the EEG change. The pO_2 change during arousal was interpreted as due to an acute increase of the cortical oxygen consumption which was secondarily modified by concomitant local cortical vasomotor reactions. The results support the view that the cortical oxygen tension is stabilized by homeostatic mechanisms which adapt the blood flow to the functional demands. When the functional demands are greater than the supply, the pO_2 level falls. This was especially evident during epileptic seizures, when the pO_2 level fell markedly and pathologic patterns appeared in the EEG.

The relationship between the oxidative metabolism of the cerebral cortex and the EEG remains enigmatic On the one hand there are older as well as more recent studies which have shown that the regional cortical blood flow changes with alterations in the cortical functional activity (ROY and SHERRINGTON 1890, LANDAU *et al* 1955 SOKOLOFF 1961) Such blood flow changes which closely parallel general changes in the EEG (INGVAR 1958), have commonly been interpreted as due to an inherent regulation — probably by metabolites — of the blood flow to the functional activity (SCHMIDT 1950 KETY 1960) On the other hand quantitative studies of the *average* oxygen uptake rate of the whole brain have not demonstrated any significant differences between states characterized by widely different EEG patterns such as sleep the resting state awake and tense alertness (MANGOLD *et al* 1955, SOKOLOFF *et al* 1955 *cf* SOKOLOFF 1959) These studies have however all been made with techniques involving the brain as a whole Regional cortical metabolic changes accompanying shifts in EEG pattern might therefore have escaped detection It therefore seems evident that the cerebral metabolic correlates of different EEG patterns should be studied locally in the cortex itself

Polarographic techniques have been used previously for qualitative studies of variations in cortical oxygen tension concomitant with changes in cortical functional activity Thus DAVIS McCULLOCH and ROSEMAN (1944) and DAVIES and REMOND (1946) demonstrated a lowering of the cortical oxygen tension during epileptic seizures MEYER and DENNY BROWN (1955) also observed some relations between the EEG and the cortical oxygen availability

In the present investigation the oxygen tension of the cerebral cortex of the cat was measured with a platinum cathode of the CLARK type (LUBBERS 1960) by means of a continuous relative technique which has been described recently (INGVAR LUBBERS and SIESJO 1960) Various forms of general wide spread changes in cortical electrical activity were brought about by means of physiological pharmacological or electrical stimulation While the cortical pO_2 was found to be very stable when the EEG pattern was steady or changing slowly it was found that several forms of regular changes in the cortical pO_2 could be recorded during acute EEG changes The general conclusion is reached that such changes in pO reflect acute alterations in cortical oxygen consumption These pO_2 changes may be modified by secondary local adjustments of the cortical circulation

Preliminary reports of the findings have been presented previously (INGVAR SIESJO and LUBBERS 1959 LUBBERS 1960)

Methods

In a previous report (INGVAR LUBBERS and SIESJO 1960 a detailed description has been given of the general experimental procedures For the present investigation 18 experiments were carried out in cats of which six were anaesthetized with Nembutal initial dose 40 mg kg i p) and the remainder with ether and Pentothal In the latter

group a spinal section was made at C1 in eight cases (*encéphales isolés*) and a brain stem section at the intercollicular level in four cases (*cerveaux isolés*). In all encéphale isolé preparations and in the majority of the other experiments a curarizing agent (Flaxedil) and artificial respiration was used. The vagi and cervical sympathetic nerves were sectioned in all except two experiments.

The membrane covered platinum cathode for pO_2 tissue measurements (CLARK 1956, LÜBBERS 1960) provides a sensitive, stable and fast reacting instrument (63 % deflection reached in about 5 seconds when shifting from pure nitrogen to air) for continuous relative measurements of tissue pO_2. The active area of the several electrode models used was 3—6 mm in diameter, i.e. the electrodes recorded over an area containing both blood vessels and cortical tissue proper (see Discussion). The pO_2 electrode was usually placed on the middle suprasylvian gyrus. In some preliminary experiments the pO_2 was recorded by small naked platinum cathodes (about 0.5 mm in diameter) which were sharpened and inserted into the cortical tissue. A silver-silver chloride reference electrode was placed in muscle tissue in those cases.

Arousal reactions in the EEG were brought about by adequate stimulation (usually nociceptive) or by high frequency electrical stimulation (125—200 square wave pulses of 1 msec duration during 5—10 sec) of reticular parts of the mesencephalon and the diencephalon with stereotaxically oriented steel needles (MORUZZI and MAGOUN 1949). A GRASS stimulator with a transformer output was used. Needle electrodes were also used for stimulation of thalamic or other areas subcortically. Local stimulation of the cortical surface was made with two platinum ball electrodes.

Epileptic seizures were induced by i.v. injections of Metrazol (pentamethylene tetrazol) solutions in a concentration of 5 or 10 per cent. The cerebral oxidative metabolism, and hence the EEG, was depressed acutely by injections of a potassium cyanide solution (1 mg/ml) in amounts of 0.2 to 0.5 mg into the carotid artery through a cannula in the central end of the cut lingual artery. The effects of intravenous or intracarotid injections of Nembutal or Pentothal (1—15 mg/kg) upon the EEG and cortical pO_2 were also studied.

In 5 experiments relative measurements of the cortical blood flow were obtained either by the technique of INGVAR and SÖDERBERG (1956) or by means of a thermistor placed subdurally a few mm from the pO_2-electrode. The cortical blood flow variations, the EEG, the blood pressure (measured with an electromanometer (ELEMA, Stockholm) through a cannula in the femoral artery) and the pO_2 changes were all recorded graphically by means of an OFFNER type D3 electroencephalograph (cf. I[illegible] AR, LÜBBERS and SIESJÖ 1960).

Results

With stable systemic circulatory conditions and controlled respiration the cortical pO_2 measured was found to vary very little (less than 5 % as a rule, cf. INGVAR, LÜBBERS and SIESJÖ 1960). The cortical pO_2 was not only found to be stable in states characterized by a uniform EEG pattern such as during anaesthesia but also during states accompanied by gradually changing EEG pattern. For example during the disappearance of anaesthesia and its effects in the EEG or during the slow induction of an arousal pattern in the EEG by small doses of Metrazol the cortical pO_2 did not change appreciably provided concomitant effects upon the systemic circulation could be avoided.

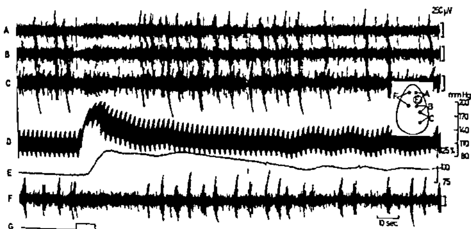


Fig 1 Cat Light Pentothal anesthesia with Flaxedil Artificial respiration. Vagi and cervical sympathetic nerves sectioned
Records of EEG (*A* *B* *C* and *F*) blood pressure (*D*) cortical surface pO_2 (*E*) At signal (*G*) the mesencephalic reticular formation was stimulated electrically (3 × threshold intensity for EEG arousal 250 pulses per sec 1 msec duration) Concomitant with the EEG arousal reaction there was a marked pressor response (*D*) which increased the cerebral blood flow and thus gave rise to an increase of the cortical surface pO (*E*) Note similar lengths of blood pressure and pO reactions

Acute alterations of the EEG on the other hand were regularly found to be accompanied by cortical pO_2 changes Some such changes which were studied systematically will now be described

I *Cortical pO_2 during EEG changes elicited by electrical stimulation*

The main interest was directed toward cortical pO_2 changes accompanying the arousal reaction (cortical activation or desynchronization) of the EEG This reaction was studied in 15 cats (8 *encéphales isolés* 4 *cerveaux isolés* and 3 Nembutal preparations) displaying a synchronized EEG with spindles

In preparations with an intact brain stem the electrically induced arousal reaction is in most cases accompanied by a pressor response (HODES and MAGOUN 1942) which may outlast the activation period in the EEG Such an increase of blood pressure is followed by an increase in cerebral blood flow and hence the cortical oxygen tension is also influenced (Fig 1) Due to the systemic circulatory changes it is impossible to investigate local inherently cortical changes in blood flow and pO_2 during arousal in such animals

In *encéphale* or *cerveau isolé* preparations however the above described pressor response in arousal is eliminated Furthermore if a curarizing agent and artificial respiration is used any respiratory changes or motor reactions caused by the brain stem stimulus will be excluded It should also be pointed out that in *cerveau isolé* preparations a brain stem stimulation does not affect the vasomotor control of cerebral vessels (HOLMQUIST INGVAR and SIESJO 1957) Preparations of this type are thus suited for studies of the relationship between

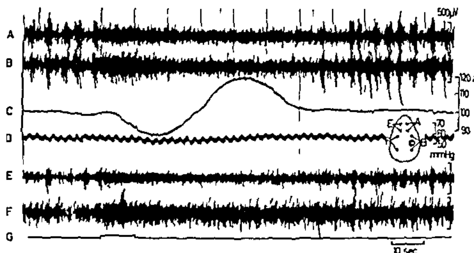


Fig 2 Cat *Encéphale isolé* preparation with Flaxedil Artificial respiration Vagi and cervical sympathetic nerves sectioned
Records of EEG (*A* *B* *E* and *F*) cortical surface pO_2 (*C*) and blood pressure (*D*) At signal (*G*) the *mesencephalic reticular* formation was stimulated electrically (3 × threshold, 250 pulses per sec 1 msec *duration*) *Note* absence of pressor response (*cf* Fig 1) and the biphasic pO_2 reaction concomitant with the EEG arousal

the cortical functional activity and changes in the oxygen tension in the cortical tissue It should be emphasized however that in comparison with preparations with an intact brain stem those with a brain stem section or, especially those with a spinal section at CI showed a lower blood pressure (60 to 100 mm Hg as compared to 80—150 mm Hg see Discussion)

In all *encéphale* or *cerveau isolé* preparations the EEG effect during arousal was thus found to be accompanied by an initial small decrease in the cortical pO_2 measured followed by a secondary increase (Fig 2) The latter was usually by far the more regular one and the most pronounced one The initial decrease started simultaneously with the stimulation or with a latency of one to two seconds The increase when present alone usually had a latency of 10 seconds or more Expressed in terms of percentage taking the resting cortical pO_2 during air breathing as standard the initial phase of decrease seldom reached below 90—95 per cent while the phase of increase often reached 120 to 125 per cent or more The typical pO_2 change in arousal could be elicited from all structures which gave a wide spread desynchronization of the EEG Thus structures pertaining to the reticular system in the mesencephalon (stereotaxic coordinate frontal minus 2 and forward), as well as in the diencephalon (up to plus 11) were stimulated with essentially the same result

The change in cortical pO_2 during arousal like the EEG change, was grossly proportional to the intensity of the stimulus (Fig 3A) The duration of the initial decrease of pO_2 varied with the state of oxygenation of the arterial

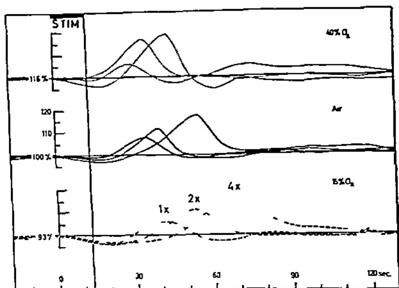


Fig 3 A.

Fig 3 Diagrams showing different cortical surface pO_2 reactions during electrical stimulation of the mesencephalic reticular formation The individual curves plotted directly from original records *Encéphale isolé* preparation as in Fig 2

A Cortical surface pO_2 reaction during arousal at different stimulus intensities (1 ×, 2 × and 4 × threshold for EEG arousal respectively) and with different oxygen concentrations in the inspired air Note relationship of pO_2 change and stimulus intensity Note also lengthening of initial phase of decreased cortical pO_2 with low oxygen respiration and shortening with high oxygen respectively

blood Thus the initial period of decrease was lengthened when the preparation had been allowed to breathe a gas mixture with less oxygen than in air, while it was shortened or completely eliminated in hyperoxia. The height of the secondary phase of increased pO_2 did not change appreciably when the level of oxygenation varied while the form of this wave underwent smaller alterations (Fig 3A)

It was also possible to alter the cortical pO_2 effect during arousal by changing the rate of ventilation Hyperventilation which in itself often caused a small reduction of cortical pO_2 gave a general diminution of the deflections in arousal while the opposite hypoventilation caused a marked increase of the initial downward deflection Both procedures could be repeated with the same result at various levels of oxygenation (Fig 3B)

It is of interest that in hypoventilation (*i e* mild hypoxia and asphyxia) of a degree not affecting the resting EEG repeated brain stem stimulations gave rise to very marked initial phases of decrease of cortical pO_2 which could last for minutes (Fig 3B I and II) In such states the brain stem stimulus did not elicit a regular activation with desynchronization of the EEG Instead after only a very short period of high frequency EEG waves there

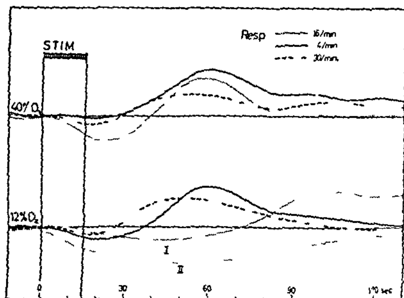


Fig 3 B

B Influence of respiratory rate upon cortical surface pO_2 reaction during arousal in hyperoxia (40 % O_2) and hypoxia (12 % O_2)
Note diminution of total height of reaction with hyperventilation (dashed lines) and enhancement of initial hypoxic phase with hypoventilation (dotted lines) When two arousal reactions (I and II) were elicited in immediate succession in the hypoxic (12 per cent oxygen) and hypoventilated state the cortical pO_2 records showed excessively longlasting hypoxic deflections. In the last case (II) the EEG showed slow waves instead of the regular arousal pattern (See text)

sometimes developed a general slow wave pattern lasting until the return of the cortical pO_2 to the resting level

Records of cortical blood flow again demonstrated that the EEG arousal reaction is accompanied by an increase in the local cortical blood flow which lasts as long as the desynchronization of the EEG and which is unrelated to simultaneous changes in the systemic circulation (INGVAR 1955 INGVAR and SÖDERBERG 1956 INGVAR 1958) The onset of the increase in cortical blood flow started about 3—8 seconds after the onset of the stimulus which induced the arousal reaction The increase in blood flow paralleled the increase in pO_2 There was in no case evidence of a diminution of blood flow initially in the arousal period when the cortical pO_2 often showed a small transient decrease Such a diminution of flow may however, often be seen after brain stem stimulation in non curarized preparations with an intact brain stem and intact cervical sympathetic nerves (INGVAR 1955 GEIGER 1958)

Arousal reactions induced by physiological stimulation were also recorded. In the preparations used these reactions were, however unstable and never as pronounced and longlasting as those elicited electrically Consequently the pO_2 changes recorded were small and not suitable for a systematic study

Local electrical stimulation of the cortex was made in two experiments with a pair of electrodes placed on both sides of the pO_2 cathode It gave rise to an immediate diminution of the cortical pO which was roughly proportional to the intensity of stimulation Especially large pO_2 decreases were obtained with stimulation intensities producing a local epileptic after discharge It was felt, however that further studies with local cortical stimulation would require refinement of the techniques used

Thalamic stimulation (which was made in three experiments) which gave a recruiting response of the classical type (MORISON and DEMPSEY 1943) was found to give a small increase in the pO_2 measured This increase initially amounted to only a few per cent and later slowly disappeared even if the stimulus was maintained This response was more widespread than similar small ones elicited regionally in the cortex by *e g* transcallosal stimulation (in an area homologous to the stimulation point) or by stimulation of specific projection nuclei in the thalamus A systematic exploration of the topography of cortical pO_2 effects was however outside the scope of the present investigation

II *Chemically induced changes in cortical pO_2*

By suitable chemical agents the cerebral respiratory rate can be either enhanced or depressed In the present experiments Metrazol, barbiturates and potassium cyanide were used (*cf* MCILWAIN 1959)

When administered carefully in fractionated doses Metrazol gives rise to a desynchronization in the EEG Like an electrically induced arousal such a pharmacologic arousal is accompanied by an increase in cortical blood flow (INGVAR 1958) Like the EEG effects this increase of flow is much more long lasting than the transient effects elicited electrically It was found that the Metrazol arousal when induced slowly was not accompanied by any substantial change in the resting cortical pO_2 Some transient effects could be correlated with systemic circulatory effects immediately following each injection Even in extreme degrees of cortical activation there was only a small increase if any change at all in the pO_2 provided an epileptic seizure did not occur

With higher doses of Metrazol or following a sudden injection of a larger amount a series of epileptic seizures was induced Such seizures either appeared spontaneously or could easily be elicited by afferent somato-sensory or auditory stimulation It was then possible to confirm the observations of DAVIS MCCULLOCH and ROSEMAN (1944) and of DAVIES and REMOND (1946) who also found a decrease in the cortical oxygen tension during epileptic seizures

Attention was directed to the time sequence of the strikingly regular cortical events during epileptic seizures induced in *encéphale* or *cerveau isolé* preparations In such preparations there is no interference by systemic circulatory respiratory

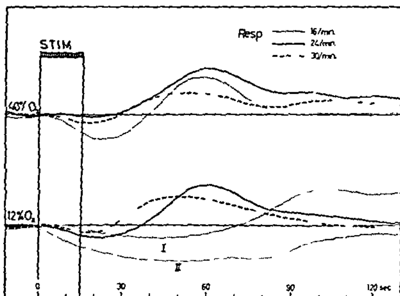


Fig. 3 B

B Influence of respiratory rate upon cortical surface pO reaction during arousal in hyperoxia (40 O_2) and hypoxia (12 , O_2)
Note diminution of total height of reaction with hyperventilation (dashed lines) and enhancement of initial hypoxic phase with hypoventilation (dotted lines). When two arousal reactions (I and II) were elicited in immediate succession in the hypoxic (12 per cent oxygen) and hypoventilated state the cortical pO records showed excessively longlasting hypoxic deflections. In the last case (II) the EEG showed slow waves instead of the regular arousal pattern (See text)

sometimes developed a general slow wave pattern lasting until the return of the cortical pO_2 to the resting level

Records of cortical blood flow again demonstrated that the EEG arousal reaction is accompanied by an increase in the local cortical blood flow which lasts as long as the desynchronization of the EEG and which is unrelated to simultaneous changes in the systemic circulation (INGVAR 1955, INGVAR and SÖDERBERG 1956 INGVAR 1958) The onset of the increase in cortical blood flow started about 3—8 seconds after the onset of the stimulus which induced the arousal reaction The increase in blood flow paralleled the increase in pO_2. There was in no case evidence of a diminution of blood flow initially in the arousal period when the cortical pO_2 often showed a small transient decrease Such a diminution of flow may, however often be seen after brain stem stimulation in non curarized preparations with an intact brain stem and intact cervical sympathetic nerves (INGVAR 1955 GEIGER 1958)

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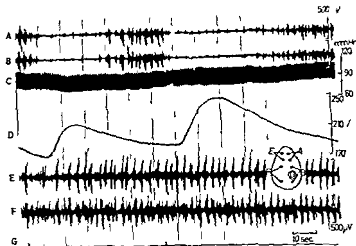


Fig 5 Cat Nembutal anesthesia.
Records of EEG (A B E and F) blood pressure (C) and cortical surface pO_2 (D) Sample record taken from period during which small doses of potassium cyanide were injected into *the right carotid artery First signal (C) marks injection of 0 2 mg* (in 0 2 ml saline of 38 C) second signal injection of 0 4 mg (in 0 4 ml)
Note right sided depressions of EEG (A and B) following the injections These depressions were accompanied by acute rises in cortical surface pO_2 due to acute diminution of oxygen consumption. (The high relative level of cortical pO as well as the initial slope in the oxygen record were caused by preceding injections of cyanide and other drugs)

in Fig 4 from an experiment with a naked platinum cathode and simultaneous cortical blood flow measurement

Finally the experiments with potassium cyanide and barbiturates should be summarized When cyanide was injected into the carotid system on one side a homolateral depression of the EEG was obtained within a few seconds and, following it there was a homolateral increase in cortical pO_2 (Fig 5) Records of cortical pO_2 and EEG contralaterally did not show any substantial changes The magnitude of the cyanide effects upon the cortical pO_2 showed a clear relation to the dose given Similarly, *following intracarotid injections of 1—2* mg of Pentothal a homolateral increase (about 5 %) in cortical pO_2 could be obtained together with the homolateral development of a typical barbiturate EEG pattern both changes having about the same duration The effect of barbiturates administered intravenously were obscured by concomitant blood pressure changes which affected the cortical blood flow

Discussion

In the present experiments it has been assumed that the pO_2 electrodes used *gave a relative measurement of the mean tissue* pO_2 *in the cerebral cortex* This assumption has recently been strengthened by a series of experiments in dogs (GLEICHMANN *et al* 1962) with a *quantitatively* measuring pO_2 electrode (LÜBBERS 1960) Such quantitative measurements on the cerebral cortex in

dogs in a steady state showed a fair agreement between the experimentally measured "surface" pO_2 values and the mean tissue pO_2 calculated from an oxygen diffusion model for cortical gray matter (THEWS 1960) It is therefore possible to derive some general conclusions from the results presented above concerning the relation between the functional activity and the mean oxygen tension of the cerebral cortex.

The stability of the cortical pO_2 measured, not only in steady states but also when slow gradual changes took place in the EEG, might be interpreted as further evidence for the existence of homeostatic mechanisms in the cortical tissue which secure an adequate pO_2 by adjusting the blood flow to the functional activity Investigations which demonstrate a parallelism between cortical blood flow and degree of activation of the EEG were mentioned in the introduction.

Discussing the cortical pO_2 effects recorded during electrically induced acute EEG changes such as arousal reactions it should be emphasized that these reactions were studied systematically in preparations in which the systemic circulatory and respiratory accompaniments of the arousal had been eliminated deliberately by brain stem or spinal sections Many of the *encephales* and *cerveaux isoles* also had a low blood pressure When in an isolated brain of this type the electric stimulation suddenly changes the state of rest into one of as it seems extreme activation the situation is indeed artificial. The conclusions reached in the arousal experiments cannot therefore be immediately applied to conditions in normal brains which undergo changes from sleep to wakefulness under the full "protection" of circulatory and respiratory adjustments in arousal (*cf* MANGOLD *et al* 1955 SOKOLOFF *et al* 1955) However without this "protection" it was shown in most cases that a typical arousal was initially accompanied by a decrease of cortical pO_2 Like the subsequent regular increase in pO_2 the initial decrease was found approximately proportional to stimulus intensity and the EEG effect.

Since the initial decrease in pO_2 was never found to be accompanied by a reduction of the cortical blood flow we conclude that it was caused by an acute increase in the cortical oxygen consumption concomitant with the activation of the EEG This conclusion is further strengthened by the fact that the decrease in pO_2 was very much enhanced by hypoxia and shortened or eliminated in hyperoxia.

The conclusion above would imply that the increase in cortical oxygen consumption should last as long as the period of cortical activation. However cortical arousal is accompanied by a local increase in the cortical blood flow (see above) This increase with all likelihood explains the ensuing phase of increase in cortical pO_2 which usually lasted as long as the activation period in the EEG Simultaneous records of cortical blood flow and cortical pO_2 paralleled each other closely except, as mentioned in the initial period of decreased pO_2

The interpretation given above is further supported by the finding that the cortical carbon dioxide tissue tension (see SIESJO 1961) (cortical pCO_2) during arousal shows an increase which lasts as long as the activation period in the EEG i e the cortical pCO_2 parallels the blood flow record (INGVAR, SIESJO and HERTZ 1959) In other words in spite of an increase in cortical blood flow which tends to lower the tissue pCO_2 and increase was found This proves that there is an increased production of carbon dioxide during the period of arousal There is then ample experimental evidence for the view that the reticular activating system by its influence upon the cortical functional activity as seen in the EEG also influences the oxidative metabolism of the cerebral cortex and that this secondarily influences cerebral blood flow (INGVAR 1958)

The diphasic pO_2 change during electrically induced arousal reactions would seem to be related to the also diphasic DC potential shift concomitant with arousal reactions (GOLDRING and O LEARY 1951 ARDUINI 1958 VANASUPA et al 1959) It remains for future investigations to clarify whether shifts in dendrite membrane potential takes place concomitant with changes in cortical oxygen consumption induced from the brain stem.

There are two observations above which especially illustrate the cortical events during critical conditions of oxygenation In both instances situations were created in which the available oxygen apparently did not suffice to meet the functional demands of the cortical tissue

The first observation concerns brain stem stimulation during hypoventilation when the resulting hypoxia was not quite sufficient to alter the resting EEG Such stimulations were followed by a long lasting decrease in pO without any secondary increase and accompanied by slow waves in the EEG instead of the regular activation pattern seen at normal levels of oxygenation It is known that hypoxia as well as an increase in blood pCO_2 both present in hypoventilation give rise to cerebral vasodilation (NOELL and SCHNEIDER 1944 KETY and SCHMIDT 1948 SCHNEIDER and OPITZ 1950) Consequently when the cortical functional activity is increased during hypoventilation any further adjustment of the blood flow must be limited This would explain the absence of the secondary increase of cortical pO_2 in such situations

The appearance of slow waves in the EEG during arousal reactions in a hypoxic state merits further study This type of EEG response is most likely related to similar effects of brain stem stimulation described by MORISON FINLEY and LOTHROP (1943) INGVAR (1955) and by INGVAR and SODERBERG (1958) Probably this response should be differentiated from other synchronizing effects upon the cortex elicited in the lower brain stem (MAGNES MORUZZI and POMPEIANO 1961)

The second observation concerns the epileptic seizure Here the cortical pO_2 regularly showed a substantial decrease (up to 25 %) which always started a few seconds after the onset of the high voltage epileptic activity in the EEG

In simultaneous records of the cortical blood flow it was shown that the cortical pO_2 started to decrease in spite of the fact that the blood flow was already increased. In fact the lowest pO_2 levels were reached after the blood flow had already reached its maximum. As soon as the seizure stopped the pO_2 rose again increasing to a maximum during the postictal phase of electrical silence during which as a rule the blood flow was still substantially augmented. It was thus confirmed that the epileptic seizures were never initially accompanied by a reduced cortical blood flow (JASPER and ERICKSON, 1941). The dramatic decrease of cortical pO_2 which developed after the great augmentation of the cortical blood flow must be explained by the greatly increased cortical oxygen consumption during the seizure as shown in the monkey (for the whole brain) by SCHMIDT, KETY and PENNES (1945). It should be recalled here that the cortical pCO_2 also shows a dramatic increase following an epileptic seizure (INGVAR, SIESJÖ and HERTZ, 1959). The present results therefore indicate that during the generalized epileptic seizure a state of relative oxygen deficiency in the cortex results due to the fact that the local cortical blood flow, already substantially augmented, cannot adequately compensate for the great increase in oxygen consumption (JASPER and ERICKSON, 1941; GÄNSHIRT et al., 1959). The results from local cortical stimulations in which local epileptic "after discharges" were produced indicates that the interpretation given also holds true in such cases. It remains, however, for future quantitative measurements to establish the extent and degree of the above postulated relative oxygen deficiency in the cortical tissue during the epileptic discharge.

Note added to proof. In a recent communication KANZOW et al. (KANZOW, E., D. HELD and J. RICHTERING: Beziehungen zwischen EEG-aktivierung und Durchblutung der Hirnrinde des Hundes. Pflug. Arch. ges. Physiol. 1960/61, *272*, 13.) have reported that EEG activation in non anesthetized dogs is not always accompanied by an increase of cortical circulation. These workers measured the cortical circulation locally by means of a thermoelectrical method. Their results emphasize the great variability in local cortical blood flow in the non anesthetized state which, as pointed out above, cannot directly be compared with the conditions used in the present experiments.

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Acta physiol. scand. 1962 55 225—230

From the Department of Pharmacology University of Gothenburg Gothenburg Sweden

Lactic Acid Production and Adrenaline Reversal in Experiments on Isolated Smooth Muscle

By

Ella Mohme Lundholm

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Abstract

Mohme Lundholm E. *Lactic acid production and adrenaline reversal in experiments on isolated smooth muscle* Acta physiol scand. 1962 55 225—230 — In experiments on isolated muscle preparations from rabbit stomach adrenaline had a contracting effect and increased the lactic acid content. Lactic acid was determined with an enzymatic method After treatment with dibenamine the preparations were relaxed by adrenaline The lactic acid production remained unchanged Adrenaline reversal after dibenamine was interpreted as a selective inhibition by dibenamine of the contracting effect of adrenaline, following which the relaxing and lactic acid stimulating action was evidenced

Studies of the relaxing effect of adrenaline on smooth muscle have demonstrated a close correlation between that effect and an increase in the lactic acid content of the muscle (Mohme Lundholm 1953 1956 1957) Stimulation of the lactic acid production has also been demonstrated in such smooth muscle as is contracted by adrenaline (Lundholm and Mohme Lundholm 1962) — It was nevertheless possible under certain experimental conditions — *e g* after administration of adrenolytic agents — to block selectively the contracting effect of adrenaline without inhibiting the lactic acid production (Lundholm and Mohme Lundholm 1960) No relaxing effect occurred however in those experiments — probably because the tonus level of the tissue preparations was low The following report is concerned with experiments on rabbit stomach muscle from which it emerged that adrenaline reversal following dibenamine was associated with an elevation of the lactic acid content of the muscle



Fig 1 Isolated rabbit ear perfused with Tyrode solution Flow measured with drop recorder 1 Adrenaline 0 1 μg in 0 2 ml 0 9 NaCl at pH 3 B Same ear perfused with benzylimidazoline 0 2 mg ml At arrow 2 adrenaline 0 3 μg in solution at pH 3 At arrow 3 adrenaline 0 3 μg in Tyrode solution at pH C As in B but showing the effect of 0 2 ml 0 9 NaCl at pH 3 — It is evident from the diagram that although the contracting effect of adrenaline was not totally suppressed by benzylimidazoline the acid solvent was nevertheless able to induce dilatation Not even when the contracting effect of adrenaline had been completely inhibited by benzylimidazoline however did reversal occur after adrenaline in neutral solution

Method

A rabbit weighing between 2 and 3 kg was killed by air embolism The stomach was removed opened along the lesser curvature and rinsed out with Tyrode solution. The muscle coat was carefully stripped of mucosa and connective tissue From the muscle coat in the fundus portion were taken specimens 15 mm wide 20—25 mm long 0 8—1 0 mm thick, and weighing 0 3—0 4 g Each preparation was attached along the sides in a small plastic frame so as to ensure a uniform load over the entire surface The changes of length in the longitudinal axis of the circular muscle fibers were recorded by an isotonic pen with a load of 5 g and with a ratio of 1 10 The muscle coat was thickest towards the lesser curvature side and this was allowed for by taking as control and test preparations two contiguous pieces of muscle with as uniform a thickness as possible It was found that the lactic acid content was higher in thick preparations Each mounted preparation was transferred to an organ bath containing 40 ml Tyrode solution with 0 1 per cent glucose at 38 C This solution was aerated with 93 5 % O_2 and 6 5 % CO_2 whereby it assumed a pH of 7 15 Two of the preparations were first treated for 20 min with dibenamine at a concentration of 1 10^{-6} The Tyrode solution in all organ baths was then changed To increase the tone of the dibenamine treated preparations carbacholine in a concentration of 1 5 10^{-8} was added When after 10—15 min the tone had reached a constant level freshly prepared neutralized adrenaline solution was added in a concentration of 2 5 10^{-6} to one untreated and to one dibenamine carbacholine treated preparation After 1—9 min, when the effect of adrenaline was distinct but not maximal the adrenaline treated preparations were removed from the organ baths and frozen in difluordichlormethane (freon 12) containing carbon dioxide snow at — 75 C

The preparations were dried rapidly on filter paper weighed on a torsion balance and immersed in small test tubes containing 1 ml iced 3 per cent perchloric acid. They were then homogenized with the use of a miniaturized modification of the Ultra Turrax homogenizer (manufactured by JANKE and KUNKEL) The extract was twice centrifuged for 30 min at 3 000 g after which the clear solution was chilled to — 20 C Lactic acid assay was as a rule carried out the following day

The extracts were assayed with the aid of L(+)lactic acid dehydrogenase (BOEHRINGER) ad modum PFLEIDERER and DOSE (1955) but with that modification of the buffer solution which was reported by SCHOLZ *et al* (1959) In matched 10 mm quartz cuvettes 0 1 ml of the measured extract was added to a solution consisting of 2 ml buffer with 0 5 M glycine and 0 4 M hydrazine pH 9 5 0 2 ml 0 027 M diphosphopyridine nucleotide (DPN) and 0 03 ml L(+)lactic acid dehydrogenase (2 mg enzyme protein

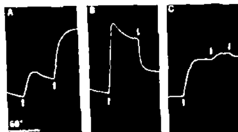


Fig 2 Rabbit stomach muscle Tyrode solution with 0 1 glucose. A At the first arrow carbacholine 2 5 10⁻⁹ at the second arrow adrenaline 0 5 10⁻⁶ B The preparation has earlier been treated for 20 min with dibenamine 1 10⁻⁴ At the first arrow carbacholine 7 5 10⁻ at the second arrow adrenaline 2 5 10⁻⁶ C The preparation treated with dibenamine At the first arrow carbacholine 2 5 10⁻ at the second arrow 0 005 M sodium carbonate at the third arrow adrenaline 2 5 10⁻⁶

per 1 ml) A blind test in which 3 per cent perchloric acid was substituted for the extract was carried out in another cuvette Following incubation for 60 min in a water bath at 25 C the extinction difference at 340 mμ and 25 C was determined in a thermostat regulated Beckman DK2 recording spectrophotometer An extinction difference of 0 1 was equivalent to 0 161 μM L(+)lactate (33 8 μg/ml extract) As a control the lactic acid content was simultaneously determined in a solution which contained a known quantity of zinc L(+)lactate

In repeated lactic acid assays of a solution containing a known amount of lactate the maximum yield varied between 102 and 107 per cent Zinc L(+) lactate added to the extracts could be quantitatively recovered A comparison between this and other methods for the determination of lactic acid will be published separately

Results

There are reports in the literature of numerous smooth muscle preparations in which reversal has followed addition of adrenolytic agents (for review vide BOVET and BOVET NITTI 1948 p 298) — Duplication of such experiments proved to be a formidable task A possible source of error lay in the use of acid adrenaline solutions Fig 1 shows that pseudo adrenaline reversal was induced in isolated rabbit ear after benzylimidazoline and that the effect may have been attributable to the acid solvent for adrenaline

FURCHGOTT (1959) however called my attention to the fact that in experiments on isolated stomach muscle from rabbit an unequivocal adrenaline reversal could be produced after dibenamine — even when adrenaline was added in completely neutral solution

It is evident from Fig 2 A that adrenaline at a concentration of 2 5 10⁻⁶ contracted rabbit stomach muscle and that this effect was added to the likewise contractile action of carbacholine in a concentration of 7 5 10⁻⁸ When treated with dibenamine in a concentration of 10⁻⁴ for 20 min carbacholine contracted muscle was relaxed by adrenaline (Fig 2 B)

The effect of adrenaline on the lactic acid content of the preparations is shown in Table I With contraction of the muscle by adrenaline the lactic acid content had after 1—2 min increased from 16 to 20 mg per 100 g tissue or 25 per cent In the dibenamine carbacholine treated preparations the lactic acid content was 15 7 mg per cent and the increase after adrenaline 4 2 mg per cent

Table I Effect of adrenaline on the lactic acid content of muscle strips from rabbit stomach. Lactic acid content in mg per cent

n = number of tests p = probability that the effect was due to chance.

Effect on tone	Control	Increase after adrenaline	Carbacholine + dibenamine	Increase after adrenaline
Contraction (n = 28)	16 1 ± 1 7	4 0 ± 1 6	—	—
	—	$p < 0\ 02$	—	—
Inhibition (n = 14)	—	—	15 7 ± 2.5	4.2 ± 1.8
	—	—	—	$p < 0\ 05$

It was possible, as it had earlier been with other smooth muscle (MOHME LUNDHOLM 1953 1957) to block the post dibenamine relaxing effect of adrenaline by means of glycolysis-inhibiting substances such as sodium fluoride (0 008 M) and glyceraldehyde (0 08 M) — The relaxing action of adrenaline was also inhibited by sodium carbonate at a concentration of 0 005 M (Fig 2 C)

Stomach muscle which had been contracted by carbacholine or dibenamine plus carbacholine was also relaxed by lactic acid The threshold concentration for this relaxing effect was 0 00055 M The lactic acid content of the bath coincidently rose by 5 mg per cent — Earlier experiments (MOHME LUNDHOLM 1953) had shown however that the increase of the lactic acid content in the muscle on addition of lactic acid was only 20—30 per cent of that in the bath These data suggested therefore that the adrenaline induced augmentation of lactic acid in the muscle was of such magnitude as to presuppose a relaxing effect

The above results testify to the very close correlation between adrenaline's relaxing effect and its effect on the carbohydrate metabolism.

Discussion

At the concentration employed, dibenamine blocked selectively the contractile effect of adrenaline on stomach muscle without influencing the lactic acid stimulating action while concomitantly a reversal of adrenaline's effect became manifest A plausible explanation of this reversal is that prior to dibenamine adrenaline stimulated both the contractile mechanism and the carbohydrate metabolism but that its contractile effect obscured the relaxing effect of the lactic acid production When however, the contracting effect had been selectively blocked by dibenamine the lactic acid stimulating and relaxing action became apparent This explanation is consonant with the hypothesis for adrenaline reversal formulated by DALE (1906) According to DALE there exist two kinds of adrenergic receptors in smooth muscle of which the excitatory is blocked by adrenergic blocking agents unmasking the effect of the inhibitory receptors

BULBRING and coworkers (BÜLBRING 1960 AXELSSON BUEDLING and BÜLBRING 1961) have presented additional experimental evidence of the correlation between adrenaline s relaxing action and its effect on the carbohydrate metabolism According to BÜLBRING however it is the increased energy production associated with stimulation of the carbohydrate metabolism that has an important bearing on the relaxing effect. As a result of increased energy production it is argued the sodium pump would be stimulated and the cell membrane hyperpolarized, and this in turn would lead to relaxation The increased elimination of radioactive ^{24}Na after adrenaline administration lends support to this view (AXELSSON BLEDING and BULBRING 1961)

It is at the present stage not possible to state with certainty whether stimulation of the carbohydrate metabolism produces relaxation via an augmented energy production or an increased lactic acid production or alternatively via an unknown mechanism Conflicting with BÜLBRING s interpretation however is the fact that adrenaline relaxes smooth muscle which has been completely depolarized by K_2SO_4 (EVANS SCHILD and THESLEFF 1958) and in which the sodium pump can hardly be operative

The increased sodium elimination after adrenaline can possibly be regarded as a consequence of heightened elimination of lactic acid from the muscle According to SHANES (1958 p 143) it would seem that organic acids can pass through the cell membrane only in an undissociated state Hence it is not altogether unlikely that with increased lactic acid production some of the lactate is released from the cell as sodium lactate — a phenomenon which could account for the increased sodium elimination

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From the Nobel Institute for Neurophysiology, Karolinska Institutet, Stockholm 60, Sweden

The Pause During Contraction in the Discharge of the Spindle Afferents from Primary End Organs in Cat Extensor Muscles

By

RAGNAR GRANIT and J. P. VAN DER MEULEN[1]

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Abstract

GRANIT, R. and J. P. VAN DER MEULEN. *The pause during contraction in the discharge of the spindle afferents from primary end organs in cat extensor muscles.* Acta physiol. scand. 1962. 55. 231—244. — Recently much detailed information has become available about the structural design of the muscle spindles. It seems highly pertinent that the large spindle afferents from primary end organs possess two kinds of terminals, one set deriving from the nuclear bag of special nuclear bag fibres, another from purely muscular short so-called nuclear chain fibres. Also spindles may vary in length over more than a tenfold range (cat). The pause in the discharge of the muscle spindles in isometric contraction has been studied and the findings evaluated from the point of view of spindle anatomy. The primary end organs were found to fall into two groups, long pause spindles and short pause spindles. This grouping is assumed to express their anatomical length. This may vary between 2 and 22 mm. The pause either ended with a phasic burst, often succeeded by a brief secondary pause, or else the discharge was resumed in a tonic fashion. Thus spindles were phasic or tonic with respect to how they resumed firing after the pause. Nearly all the tonic spindles were found among the long pause spindles. The hypothesis adopted to explain the phasic-tonic differentiation is that phasic spindles are dominated by nuclear bag fibres, tonic ones by nuclear chain fibres. Spindles are also known to vary considerably with regard to the relative number of these two different fibre types.

[1] Visiting Special Trainee of the National Institute of Neurological Diseases and Blindness, Bethesda, Md., U.S.A.

B H C MATTHEWS (1933), who discovered the pause in the discharge of the muscle spindle afferents during contraction also gave it an explanation that has been generally accepted Despite isometric recording the tendinous portion of the muscle elongates in contraction and allows some shortening (1—2 mm) of the extrafusal fibres The intrafusal fibres of the spindle being situated in parallel are thereby unloaded and their stretch receptors become silenced as had been anticipated by FULTON and PI SUÑER (1927—28) This, no doubt, is a still valid generalization but what shall concern us below is how the primary end organs are re excited after a contraction or in other words how long the pause is and in what way the discharge recommences In this respect spindles vary a great deal and the question is what these variations suggest when they are reproducible and independent of gamma control

In the past it seemed an unprofitable task to systematize and try to explain the variations alluded to because too little was known about spindle anatomy This deficiency has now been remedied, largely owing to the work of BARKER and his associates and of BOYD work that was preceded by some pioneer studies of SYBIL COOPER and lately confirmed by SWETT and ELDRED (ref below) The new picture of the organization of the muscle spindle that has emerged from these studies is detailed enough for the first time to make a study of the pause worth while It will be easier to follow the arguments of the present paper if the salient features of spindle anatomy are given already at this stage.

It has been known for a long time and often confirmed that there are long and short spindles and also that the intrafusal fibres of one and the same spindle have different length and thickness (see e g SHERRINGTON 1894 CUAJUNCO 1927 and summaries by HINES and TOWER 1928 BARKER 1949 COOPER 1960) New is the knowledge that there are two kinds of intrafusal fibres, nuclear bag (NB) and nuclear chain (NC) fibres the latter being shorter and thinner with nuclei in chains the former identical with the nuclear bag fibres known from the classical literature The new information is found in a note by COOPER and DANIEL (1956) in papers by BARKER and CHIN (1960) BARKER and GIDUMAL (1961) BARKER and IP (1961) preceded by preliminary reports as well as in a number of brief reports by BOYD (1956 1958 1959 1960 1961) and in two papers by SWETT and ELDRED (1960 a b) The large fibre afferents previously held to terminate as annulospirals in the nuclear bag are now known also to dispatch branches to the purely muscular NC-fibres which are lacking bags This means that these afferents derive information from an NB elastic structure in series with muscular poles of more viscous contractile material as well as from short purely muscular NC-fibres and hence from two different structures provided with contractile fibres in parallel with one another The whole spindle structure is in parallel with the extrafusal fibres There is also some histological evidence (COOPER and DANIEL 1956, BOYD 1959, 1961) for differences in motor innervation of NB- and NC-fibres According to the new findings it is legitimate to differentiate between

nuclear bag and nuclear chain terminals of the primary afferents from muscle spindles

The new knowledge also means that the term nuclear bag organ is too restricted to describe the primary end organs adequately nor can they be called annulospirals because they are not always provided with annulospiral endings We have therefore returned to RUFFINI s (1897 1898—99) alternative term *primary endings* The *secondary endings* according to the same recent authors are chiefly found on the NC-fibres but also in the myotube region of the long NB-fibres None of them have terminals on the nuclear bag

The new knowledge makes it imperative to reconsider known spindle properties among them the pause in the discharge of the primaries during contraction An evaluation of the general situation (GRANIT) as well as detailed information will be found summarized in the Proceedings of the Hong Kong Symposium on Muscle Receptors (to be edited by BARKER 1962)

Methods

In the present experiments cats were used the majority of which were decerebrated by precollicular suction The remainder were anesthetized with a mixture of chloralose pentobarbitone (20 mg/kg of each I P) A laminectomy was performed exposing the spinal cord and roots from L_5 to cauda equina Single fibres in the appropriate dorsal roots (L_7 S_1) were split until single spike discharges could be recorded from spindles in the gastrocnemius or soleus muscles of the left leg These fibres gave conduction velocities in the Gr 1 afferent range as calculated from the latent period sometimes recorded photographically but often merely observed on the screen at high sweep speed

The left leg was denervated except for the medial and lateral gastrocnemius nerves which were used for stimulation at knee level and in the decerebrate preparations the right leg was denervated The soleus or gastrocnemius muscles were separated so that their tendons could be attached individually to the recording apparatus A strain gauge myograph and an inductance length recorder connected to the tendon allowed for alternate or simultaneous recording of tension or length during either isometric or isotonic contraction The muscle was pulled out by weights from 100 to 500 g on a short lever For isometric contractions the lever was clamped at the length thus attained for isotonic recording it was allowed to lift the weight Since only the early onset of the discharge was of interest in this work oscillations after complete relaxation in isotonic recording were of no importance

In some experiments gamma efferents were selectively blocked with dilute procaine solution in the manner of P B C MATTHEWS and RUSHWORTH (1957 *a* *b* 1958) For cocainization xylocaine (lidocaine) was used

Results

General remarks on the pause The ensuing descriptive sections should be read with two points of view in mind (*i*) a pause in the discharge is taken to mean that the spindle is relatively too long compared with surrounding extrafusal elements so that in isometric contraction we are concerned with the time it

takes for a spindle to become matched in length with the latter, (ii) is this matching process initiated by a phasic burst or not?

To (i) both gastrocnemius and soleus spindles fall into two groups clearly distinguishable in the extremes these are the short pause (SP) and the long pause (LP) spindles (see *e g* Fig 1) There is of course an arbitrary element in these distinctions owing to border line cases Actually, however, when the muscles are properly stretched, from some 5 to 7 mm, there has been no overlap in our material which consists of 11 LP and 10 SP soleus spindles 15 LP and 19 SP gastrocnemius spindles, in all 55 analyzed at different extensions out of 61 isolated

With double shocks exceptionally three shocks the average pause of the LP spindles in soleus was 310 msec the range from 270 to 340 msec for the SP spindles the average was 150 msec the range 100 to 200 msec In gastrocnemius the average was 180 msec with range from 130 to 300 msec for LP spindles corresponding values for SP spindles averaging 70 msec with range from 40 to 110 msec With single shocks the LP average for soleus was 220 msec (range 170—320) the SP average was 90 msec (range 58—130) for gastrocnemius the corresponding figures were LP-spindles 110 msec (90—200), SP spindles 60 msec (30—83) In general when the pause is long it also tends to be labile and influenced by muscle length and stimulus strength the shorter it is the better reproducible despite variations in extension of the muscle and stimulus strength

There was one soleus spindle which in the beginning of the experiment elonged to the SP-category with a double shock pause of 130 msec later it suddenly changed type and the pause lengthened to values around 300 msec. The others maintained their character except at extreme extensions when some spindles lost their early component and gave pauses of long duration In others the pause tended to shorten somewhat with extension (values below 5 mm extension not considered) the majority being fairly stable The values given do not refer to extremes of extension and cases when this has led to an alteration of the duration of the pause

The effect of stimulus strength whether just maximal or supramaximal proved to be of little importance with SP spindles even when brief tetani were used so as to activate gamma fibres The SP spindles on the whole had more definite time constants than LP spindles though in response to gamma activation frequency might change within the burst by which they tended to resume function With the LP spindles we were surprised to find a number of them to have longer pauses with supramaximal stimuli even when the silent period then was sprinkled with spikes owing to gamma activation. In others the pause shortened somewhat in the majority it proved to be uninfluenced We have no cue to these differences The fact that by sufficient gamma activation it is possible to fill out the pause in extended spindles (MATTHEWS 1933 KUFFLER HUNT and QUILLIAM 1951) recently again

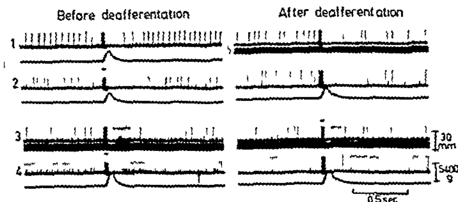


Fig 1 Pentobarbitone-chloralose intact preparation Two primary gastrocnemius afferents stimulated by brief tetanus Isometric recording sometimes with length-measuring device Muscle at 4 5 (*1* *2*) and 9 5 (*3* *4*) mm extension Spike responding in *1* and *3* became short pause phasic upon extension to 9 5 mm spike responding in *2* and *4* remained long pause and tonic The phasic spike (*1* *3*) started firing to pull at 2 mm extension the tonic (*2* *4*) fired 15—20 per sec already at zero extension The curves in a diagram plotting discharge frequency against extension were roughly parallel merely shifted by the threshold difference given Conduction velocity of phasic spike 115 m/sec of tonic spike 90 m/sec

studied by HARVEY and P B C MATTHEWS (1961 *a*) does not enter into our considerations Very strong stimuli of longer duration than we have used are required to demonstrate it More extension may be necessary with de efferented spindles than with intact ones to define their properties

To (ii) the pause either ends with a phasic burst often followed by a secondary pause and a tonic discharge or else resumption of activity is tonic from the beginning We shall speak of phasic and tonic spindles since the terms dynamic and static discharge have been defined with respect to fast stretch (*cf* KATZ 1950 HARVEY and MATTHEWS 1961 *b*) There were 51 % phasic spindles in gastrocnemius hence 49 % tonic 67 % phasic spindles in soleus hence 33 % tonic These figures refer to the total number of 61 spindles of which 37 were in gastrocnemius 24 in soleus The general tendency is for the SP spindles to be phasic and for the tonic spindles to belong to the LP category This rule held for all the gastrocnemius spindles but a few LP spindles in soleus had a phasic component Among the 55 spindles analyzed in detail there was one tonic spindle in gastrocnemius (after ventral root section) which had a short pause (SP spindle) The other tonic spindles were all LP spindles

The comparison of pause durations in isometric and isotonic contraction the former more of an internal adjustment the latter a response to stretch by the falling weight showed that with a couple of exceptions all spindles responded phasically to the pull of the weight For LP spindles the isotonic

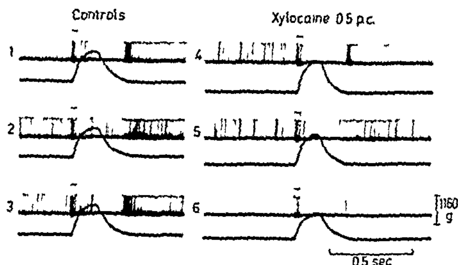


Fig 2 Decerebrate animal with good stretch reflex and spindle response to twist of pinna. Soleus spindle *1—3* repeated supramaximal stimulation by brief tetani illustrating pause partially filled out by gamma activation of spindles *4—6* gradually increasing effect of xylocaine applied below stimulating electrodes. In record *6* also some effect on alpha motor fibres. Conduction velocity of spike was 100 m/sec

values for the pause also tended to vary considerably less in duration than the isometric ones As is to be expected the SP- and LP character of a spindle less in evidence with isotonic contractions when the isometric pause was long the isotonic was definitely shorter and, vice versa, when the isometric pause was short the isotonic pause was longer or of much the same length The main role of the isotonic tests in this work has been to demonstrate that nearly all the spindles have been potentially capable of giving a dynamic response to pull and this finding adds emphasis to the question of why so many of these same spindles have not been able to respond phasically after isometric contraction

Samples analyzed Fig 1 shows two spindle afferents whose primary end organs by pressure were localized to the upper portion of the medial gastrocnemius muscle The results reported in Fig 1 were obtained both with just maximal and supramaximal stimulus strength The spindles were in the same thin filament and first recorded together Separation proved possible and the experiment was repeated by studying them alternately as in Fig 1 Records *1* and *3* belong to the first spindle (phasic), at 4 5 and 9 5 mm extension respectively, *2* and *4* to the second spindle (tonic) at the same extensions The pause of the first spindle, which had the higher threshold (see legend) was considerably shortened by extending the muscle to 9 5 mm This shortening is of an unusually high order The pause now ended with a burst of impulses

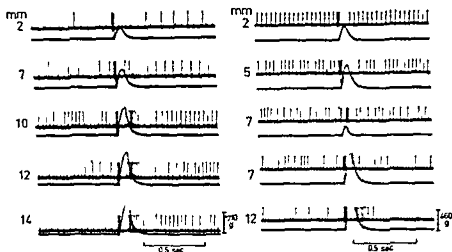


Fig 3 De efferented, chloralose pentobarbitone preparation. Two phasic gastrocnemius spindles studied at different extensions as marked on films. Third record on the right is a single shock response the others brief tetani Spindle on the left studied with submaximal spindle on the right with maximal stimuli. Five similar spindles were isolated in the same preparation Their conduction velocities were between 90 and 100 m/sec

at the turning point of contraction The secondary pause seen here is typical of the majority of spindles with an early phasic burst. The second spindle — active even at zero length — always responded tonically and resumed firing at the original rate when contraction was over even when this rate was high as in the present case (records *4*) (In record *4* there is also another phasically responding spindle, the small spike) The experiment was repeated after de afferentation (on the right) to remove a possible reflex component with preservation of whatever tonic gamma bias remained after anaesthesia (chloralose-pentobarbitone)

Fig 2 illustrates a soleus spindle under some gamma activation because in the controls a supramaximal brief tetanus was used (*1 2 3*) The pause is sprinkled with some spikes induced by a varying contribution of gamma activity Then xylocaine was applied (below the stimulating electrodes) to remove the contribution from this source (MATTHEWS and RUSHWORTH 1957 *a b* 1958) This also influenced the course of the contraction which in the intact preparation is determined by rivalry between inhibitory and excitatory impulses from stimulated muscle afferents and end organs (GRANIT 1950) The end of the pause however should be considered with respect to the end of the contraction As xylocaine successively took effect from records *4* to *6* it is seen that in spite of a very large drop in gamma bias the length of the pause changed but little when measured in relation to the falling phase of contrac

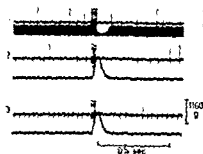


Fig 4

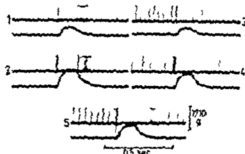


Fig 5

Fig 4 Decerebrate preparation Primary efferent ending in gastrocnemius *1* and *2* at 3 mm extension *1* isotonic (weight 100 g) *2* isometric Then spindle was activated by pinna reflex and record *3* taken. Note persistent long isometric pause and absence of phasic component even in isotonic contraction

Fig 5 Decerebrate preparation *spinal ed* at Th XII Primary afferent ending in soleus *1* single *2* double shock at 10 mm extension *3* and *4* corresponding records at 13 mm extension *5* is repetition of *4*

tion Clearly the length of the pause was a property of the peripheral instrument merely modulated by variations in 'gamma bias

Fig 3 illustrates for different extensions two gastrocnemius spindles (de efferented) starting to discharge with typical phasic bursts followed by a secondary pause As always some extension of the muscle was needed to reveal phasic component (as expected here in view of the de efferentation) For spindle on the right it should be noted that in the upper record *7* the phasic burst was emphasized by using a single shock instead of a brief tetanus This may be seen with a fast muscle like gastrocnemius The slow soleus often required a double shock or brief tetanus to show up spindle properties of the kind studied.

By contrast Fig 4 presents a gastrocnemius spindle which even isotonic contraction (record *1*) could not make phasic Record *2* gives the isometric equivalent at the same length When shifting from isotonic to isometric contraction many spindles displayed very long pauses for the first few tests with contraction This slow adjustment is not what is illustrated in record *2* which had been preceded by several tests in the isometric condition Activation of this spindle was then attempted (record *3*) and though successful to judge by the greatly increased rate of firing the discharge still started *after* contraction and in a tonic fashion

Fig 5 has been chosen to illustrate what sometimes can be seen namely that the pause greatly lengthens at great extensions It is a soleus spindle with an early phasic burst in records *1* and *2* (single and double shock) at 10 mm extension Adding 3 mm to the length of the muscle (records *3* to *5*) possibly led to some over extension of the spindle to judge by the diminution of the

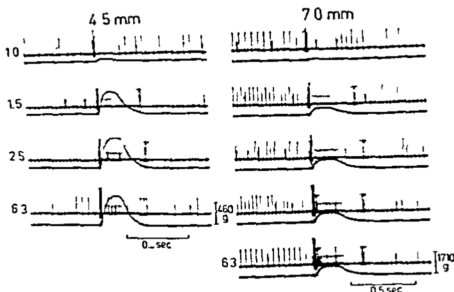


Fig 6 De efferented chloralose barbitone preparation Primary afferent ending in soleus, studied at two extensions as marked in figure Stimulus strength in multiples of lowest value on the left Activation of gamma fibres by stimuli from 2 5—6 3 times the just suprathreshold uppermost value (1 0) shows some filling in of the pause but no shift of major phasic burst relative to contraction Note tension recording sense organ filling out the pause with stronger contractions

contraction in *3* *4* and *5* to be compared with *1* and *2* respectively Record *5* is a repetition of *4* The discharge now began when contraction was nearly over Some spindles have behaved in this manner

Fig 6 shows with a soleus spindle the effect of increasing stimulus strength at two extensions 4 5 and 7 0 mm It was a de efferented animal The discharge from another sense organ a tension recording instrument sensitive to contractile tension only is seen to fill out the pause The interesting feature here is that in spite of the relatively late discharge at the end of contraction the spindle responded phasically with a secondary pause followed by the tonic component of the discharge (LP spindles in gastrocnemius have not shown up a phasic component) This mode of behaviour was very little influenced by gamma bias and extension In fact gamma activation has to be very strong to alter the fundamental features of the response obtained at the end of contraction and even then these features mostly shine through thereby supporting our general conclusion that they reflect some basic organizational principles in the design of individual spindles

This statement is best illustrated by Fig 7 records *3* and *4* (isometric but recorded with length measuring device) Strong spindle activation in record *4*

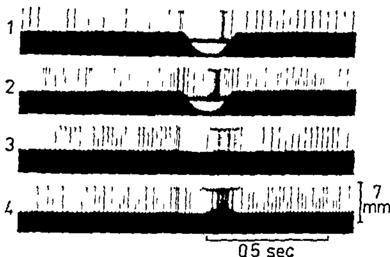


Fig 7 Decerebrate preparation *spinalized* at Th XII Primary ending in soleus pulled out by weight of 500 g to 13 mm *1* and *2* isotonic *3* and *4* isometric all taken by length recording device *1* and *3* have been recorded without special activation of spindle in *2* and *4* spindle has been activated by repeated taps on muscle itself (skin then stimulated as well) Note in *4* that despite filling out of pause the phasic burst is located much as in the control (*3*)

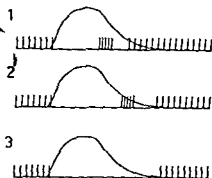


Fig 8 Schematic Diagram of the minimum of spindle types needed to account for the results obtained *1* and *2* are two versions of phasic spindles the second long pause (*LP*) *3* is the LP tonic spindle

shortened the pause if it be measured as duration of full silence by comparison with the control in record *3* But the basic length of the pause, inherent in the spindle design is clearly set off as a burst at maximal frequency in record *4* beginning in the same place there as in record *3* The pause in isotonic contraction (active re stimulation) is shown to be dependent on gamma bias in the pair *1* and *2* which are otherwise equivalent with *3* and *4* Incidentally the isotonic record *2* shows how strong gamma bias can make spindle length shift together with extrafusal muscle length so as to fill out the pause over a considerable range of shortening as concluded by ELDRED GRANIT and MERTON (1953)

Spindle types needed It is necessary to restrict a diagrammatic presentation of our findings to major types since at the moment it is hardly possible to push explanations based on spindle anatomy too far Fig 8 will have to suffice The spindle with the marked phasic component may occur in versions *1* or *2* the latter — we believe — not uncommon in soleus as it has been seen in our material of 21 soleus spindles but not among the 34 gastrocnemius spindles Type *3* is the tonic spindle which only exceptionally is of the SP variety when studied over a range of extensions The discharge in spindles of Type *1* need not necessarily start at the turning point of contraction it may be shifted downwards on the falling phase but it serves no obvious purpose to make such distinctions in a diagram of this sort

Discussion

Phasic tonic MATTHEWS (1933) in discussing the properties of primary endings with respect to sudden stretch pointed out that the initial burst of impulses underwent rapid adaptation and ascribed this to the interaction of the elastic nuclear bag with its viscous contractile polar regions The bag would respond to quick stretch by a quick extension but its tension would thereafter be released by the secondary adjustment to the new length of the pliant material in the viscous poles The initial burst of impulses and the semi stationary state of the adapted discharge have been identified (GRANIT 1955 JANSEN and P B C MATTHEWS 1961) with KATZ s (1950) dynamic and static phase of the terminal (generator) potential in frog spindles as since justified by the observations of LIPPOLD NICHOLLS and REDFEARN (1960) on similar terminal potentials in the spindles of the cat It is of considerable interest to note that the dynamic phase is absent in direct electrical stimulation of cat spindles (LIPPOLD NICHOLLS and REDFEARN 1960)

The hypothesis of MATTHEWS should to-day be confronted with the fact, first noted by COOPER (1959 published in full 1961) and then confirmed by LUNDBERG and WINSBURY (1960) HARVEY and P B C MATTHEWS (1961 *b*) and BESSOU and LAPORTE (1962) that secondary endings which are restricted to NC-intrafusal fibres and never terminate on bags possess a very insignificant if any phasic response to stretch Only primary endings have both NB- and NC-terminals (much as the large fibres from the retina pick up messages both from rods and cones) We have therefore cogent reasons for modifying MATTHEWS hypothesis to include these facts At the moment it merely states that the phasic burst on cessation of contraction is a response from the NB-component The modified version suggests that the drawn out tonic discharge is likely to be a response from the NC-component JANSEN and P B C MATTHEWS (1961) basing their argument on experiments in which they found differentiation of dynamic and static sensitivity of primaries with respect to gamma control have explained this along similar lines It is also quite in

keeping with what we know from other sense organs to find separate trans ducers for phasic and tonic responses

Let us now see what a hypothesis on such lines can do for our findings Spindles are known to vary with respect to relative number of NB- and NC-fibres BARKER and GIDUMAL (1961) report from *one* to *three* large NB-fibres and from *one* to *eight* small NC-fibres in different spindles The NC fibres which are about half the length of the NB-fibres divide somewhat twist among them selves and cross over the large fibres If the NB-fibres of a given spindle are few in number and the NC-fibres many the slack will first be taken up by the latter slow tonic structure and the discharge will be tonic Our results suggest that basic spindle design suffices to explain the differentiation described in the present work The common phenomenon of a secondary pause in phasic spindles followed by a tonic discharge suggests a change over from NB- to NC-terminals and makes it necessary seriously to consider the possibility that the NB-component never may be able to respond tonically The present results cannot of course be conclusive on this point The hypothesis also provides a satisfactory explanation for the cases in which at great extensions spindles lose their phasic response The one case in which a perfectly good spindle (in an animal useful for several hours afterwards) lost its phasic component and merely responded tonically may be due to selective loss of bias with respect to NB and NC intrafusal musculature (*cf* JANSEN and P B C MATTHEWS 1961)

Long pause — short pause The question of why matching of intrafusal to extra fusal length sometimes is slow sometimes fast concerns the relative length of t e spindle and not only whichever component responds first the NB- or the C-terminal apparatus Single spindles in the rectus femoris of the cat vary in length from 2 4 to 13 7 mm while tandem spindles run up to 22 3 mm (BARKER and IP 1961) Of the latter there is a significant number 21 % in soleus 44 % in gastrocnemius (SWETT and ELDRED 1960 *a*) In the tandem spindles the intrafusal NB-fibres run through from end to end of the whole structure Unless heavily biased by gamma activation these are likely to show up a considerable number of LP responses The spindle in Fig 4 which even in the isotonic test failed to give a phasic burst is likely to be a tandem spindle Similarly it seems likely that when there are long pauses undergoing large variations the responsive structure is likely to be a tandem spindle The longer pause of the long spindle may favour the slower NC elements One of the most striking correlations in the present material was that the tonic spindles were found among the LP spindles

The length of the pause ultimately must emerge as a compromise between anatomical length (relative to adjacent extrafusal fibres) and whatever element is dominant NB- or NC-fibres A reservation must be made here for the possi bility that some spindles are likely to possess fast motor fibres (GRANIT POM PELANO and WALTMAN 1959 *a* *b* RUTLEDGE and HAASE 1961) which means

that the spindle is co-contracted and pulled up to a shorter length together with the extrafusal fibres As a consequence the NB-fibres are ready to respond at an early moment in the turning point of contraction This reservation does not concern the principle of our explanation It merely means that such spindles are provided with a length correction operating in parallel with the changes of length in the surrounding extrafusal tissue

Our work originally began as an attempt to use the length of the pause as an index of autogenetic gamma inhibition However this line soon proved unprofitable because the great differences in the length of the pause discovered by systematic analysis persisted when the findings were controlled by de efferentation or gamma blocking by xylocaine Hence it followed that the predominant factor was peripheral and inherent in the design of the spindles and their attachment to connective tissue fascia or tendons Against this background possible minor effects of autogenetic inhibition could not be demonstrated by the index used

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Acta physiol scand 1962 55 245—254

From the Institute of Hygiene and The Nobel Institute for Neurophysiology Karolinska Institutet and The National Institute of Public Health, Stockholm 60 Sweden

Simultaneous Measurement of Blood Flow, Glomerular Filtration Rate and Urine Secretion in the Separate Kidneys of Anesthetized Rabbits

By

MATHS BERLIN

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Abstract

BERLIN M *Simultaneous measurement of blood flow glomerular filtration rate and urine secretion in the separate kidneys of anesthetized rabbits* Acta physiol scand 1962 55 245—254 — A method has been evolved for studies on anesthetized rabbits of the renal uptake extraction and excretion of substances Renal vasoconstriction which frequently occurs in rabbits during surgical procedures was partly prevented by premedication with reserpine In 11 anesthetized rabbits variations in renal blood flow glomerular filtration rate urine flow and extraction of diodrast were simultaneously studied Stability of renal blood flow glomerular filtration and urine flow could be maintained for several hours By means of changes in the blood volume or the size of the extracellular fluid space variations could readily be produced in renal blood flow and glomerular filtration rate These two variables showed strong correlation Diodrast extraction showed no large variations The right and the left kidney varied in the same way with respect to blood flow glomerular filtration rate and urine flow

For studying the renal secretion and metabolism of heavy metals it has been found advantageous to make renal artery and vein accessible for measurement and blood sampling This requires surgical measures and fairly protracted anesthesia on a suitable laboratory animal Experience from preliminary investigations suggested that rabbits can be used It has long been known however that anesthesia and surgical interference can significantly alter renal function

Earlier observations on the rabbit kidney (LAAKE 1945, WILLS and MAIN 1948 SMITH 1951) have revealed deviations during anesthesia with respect to *inter alia* blood flow and glomerular filtration Even the unanesthetized experimental rabbit can differ considerably in these respects from the intact rabbit in the resting state This is the reason why several workers (KAPLAN and SMITH 1935 DICKER and HELLER 1945, FORSTER 1947 and others) have stated that diuresis in the rabbit occurs partly by mobilization of inactive glomeruli and thereby increased filtration rate

BROD and SIROTA (1949) and FORSTER (1952) showed that if the experimental conditions do not produce renal vasoconstriction, the rabbit kidney does not differ in principle from the kidney in man dog etc In the rabbit kidney, however, the tendency to vasoconstriction is strong and may even lead to oliguria Nevertheless it seems feasible that in the anesthetized rabbit the kidneys can be brought to a stable and reproducible state in which they are suitable for experiments

In order to determine the extent to which stability and reproducibility can be obtained renal blood flow, glomerular filtration rate and urine flow were measured in rabbits anaesthetized with Nembutal and nitrous oxide The rabbits had been subjected to laparotomy in order to catheterize both ureters and the left renal vein Premedication with reserpine was given to reduce the tendency to renal vasoconstriction Variations in renal blood flow and glomerular filtration rate were studied as well as possible differences in these respects between the two kidneys by measuring the extraction and clearance of I^{131} labelled diodrast and the clearance of creatinine (KAPLAN and SMITH 1935 JOSEPHSON and KALLAS 1953) in both kidneys during brief successive periods over several hours

Methods

The experimental procedure was evolved in a large number of experiments in which the various component steps were tested and modified The final experiments were performed on rabbits of Belgian giant race weighing 4 to 5 kg The 11 experiments now to be presented were those in which the rabbits were judged from their response to the surgical procedure to be suitable test subjects The requirements were urine secretion of more than 0 1 ml per minute in each kidney and stable blood pressure When the surgical technique has been acquired these criteria can be met in at least 2 of 3 experiments The method for measuring the arteriovenous difference for isotope labelled substances in the blood is described in an other paper (BERLIN 1961), where its reliability and speed of response are discussed

Surgical Procedure

On the day before the experiments the rabbits were given 0 1 mg of reserpine (Serpedin Pharmacia) subcutaneously Anesthesia was induced by intravenous injections of pentobarbital sodium (Nembutal) and was maintained during the experiment by insufflation of a mixture of 25 oxygen and 75 nitrous oxide and repeated injections of Nembutal. The gas and air mixture was given at a rate of about 1 l/min through a fine-calibred tube in a double branched tracheal cannula

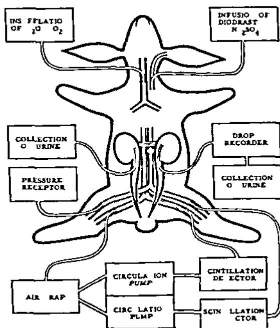


Fig 1 Arrangement of measuring and collecting apparatus

The abdomen was opened and a nylon catheter was inserted into each ureter with the tip reaching up to the renal pelvis The left renal vein was threaded *via* the left femoral vein with a polythene catheter whose outer diameter was 0 9 mm. Cannulas for measurements and infusions were introduced into the larger crural vessels and the jugular veins as shown in Fig 1 Blood loss during the operation was replaced with dextran The body temperature was kept at 37 to 38 C with the aid of light bulbs in the operating table and was registered continuously by means of an intrarectal thermistor

Osmotic diuresis was produced by infusion of 2 $^1/_2$ to 20 per cent sodium sulphate solution into the jugular vein The infusion was made with the help of compressed air All administered and excreted fluids were noted Changes in glomerular filtration rate and renal blood flow were produced by excess infusion of electrolyte or dextran during a short period Positive balance of more than 20 ml/kg body weight was not permitted

Arrangement of Measuring and Sampling Apparatus

For assessment of the renal extraction of diodrast, I [131] labelled diodrast (about 50 μC/hour specific activity 0 1 mC/mg diodrast) was infused into the jugular vein in such fashion that the arterial concentration of diodrast was kept constant as far as possible

The concentration of diodrast in arteries and the renal veins was measured with the aid of scintillation detectors through which arterial and venous blood was conducted For this purpose polythene tubes were arranged between the left renal vein (*via* the catheter) and the right femoral vein and between the aorta (*via* the left femoral artery) and the right femoral vein The rate of flow in each tube was maintained at 0 75 ml per minute by a pump (Berlin 1961) The polythene loops in the scintillation detectors contained less than 0 5 ml of blood The arterial concentration of radioactive diodrast

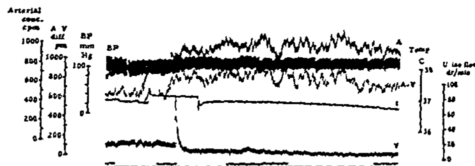


Fig 2 Typical registrations.
V = arterial concentration of diodrast in cpm
A—V = arteriovenous difference in cpm
BP = blood pressure in mm Hg
t = temperature in C
V = urine flow in drops per minute
The abscissa shows the time in minutes and the raised levels indicate time for collection of urine specimens The dots above the abscissa indicate experimental procedures such as injection or stimulation.

and the arteriovenous difference in this concentration over the left kidney were continuously registered (BERLIN 1961) The apparatus for radioactive measurements was calibrated and the background level for the scintillation detectors was registered before and after each experiment In all ratemeters the time constant was 30 sec

For measurements of clearance urine was collected from both kidneys separately in portions of 4 to 5 ml. Periods representing the collected portions were marked by the recorder The delay caused by the dead space in the catheters renal pelves and ureters was compensated by delaying the period marking for the time necessary to collect 0 5 ml urine O CONNOR and CONWAY (1922) found that in the rabbit kidney the tubuli, ter and pelvis contain on the average 0 16 ml The volume of the catheters was 0 5 ml. The urine flow on the left side was continuously registered with the aid of a drop recorder consisting of two potentiated silver electrodes The impulse evoked by the urine drop was conducted to a ratemeter

Creatinine was injected subcutaneously one hour before the clearance measurement started 10 ml of a 25 % solution were injected

Samples of arterial blood each of 0 5 ml were collected 2 to 3 times in each hour

Blood pressure was registered continuously with the aid of a pressure receptor (type Schwartzer) connected to a measuring bridge (type Ljungstrom)

All registrations were made with an eight-channel loop oscillograph (Visicorder) Fig 2 shows typical tracings

Methods of Analysis

The samples of urine were diluted to 5 ml and were frozen in polythene cylinders and stored until the radioactivity had decayed enough for measuring without coincidence losses The I^{131} activity was measured in a well crystal with a constant measuring volume of 5 ml The results were correlated with the standard solution used for calibration of the detectors.

The creatinine concentration in urine and plasma was determined according to BONSNES and TAUSSKY (1945) The error of method (coefficient of variation) in the urine tests was 5 7 per cent The values found in plasma were used to determine the exponential curve for plasma creatinine concentration during the experiment

Calculation of clearance values

The following parameters were calculated for all periods and for both kidneys

1) Renal extraction of diodrast E_D The extraction fraction in the right kidney was set as equal to that in the left kidney

$$E_D = \frac{A_D - V_D}{A_D}$$

where A_D and V_D are the concentration of diodrast in arterial and renal vein blood respectively

2) The renal blood flow RBF

$$RBF = \frac{U_D\ V}{A_D\ E_D}$$

where V is the urine flow and U_D is the concentration of diodrast in urine U_D V was obtained by determination of U_D V t (where t is the clearance period in minutes) in the well crystal U_D V being computed by division with t

3) *Creatinine clearance was calculated according to*

$$C_{Cr} = \frac{U_{Cr}\ V}{P_{Cr}}$$

where P_{Cr} is the plasma concentration of creatinine

On the basis of determinations of error of method and calculations which in part are presented elsewhere (BERLIN 1961) it was judged that the calculated values for E_D in the presented material varied within the margin of error For calculation of E_D and RBF therefore mean values bases on the E_D values for the whole material were used In calculating absolute values of RBF and E_D special consideration must be paid to the possibility of systematic errors With a confidence interval covering 95 per cent of the cases an error larger than 20 per cent should not be expectable In determining the size of changes during an experiment corresponding value however was less than 10 per cent since systematic errors here are of minor importance (BERLIN 1961)

Results

General observations

A dose of 0 1 mg of reserpine 18 hours before the experiment in rabbits of 3 5 to 4 5 kg body weight sufficed as a rule to prevent oliguria due to renal vasoconstriction and to keep the blood pressure stable at about 70 mm Hg for a long time provided that the blood volume was sustained Reserpine treated rabbits were found to be sensitive to loss of blood and fluids and meticulous substitution of all loss was necessary These precautions taken it was possible in most experiments to maintain stable blood pressure and diuresis for several hours Increase in renal blood flow and glomerular filtration rate was readily produced by increasing the fluid infusion or by expanding the blood volume with intravenous dextran

Infusion of adrenaline or noradrenaline produced persistent oliguria in the reserpine treated animals

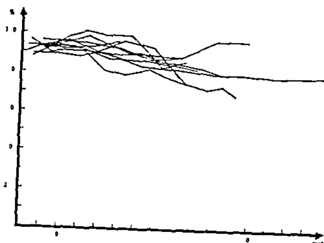


Fig 3 Extraction of diodrast by the left kidney The values are ratio A—V difference to arterial concentration of diodrast Each graph represents one experiment

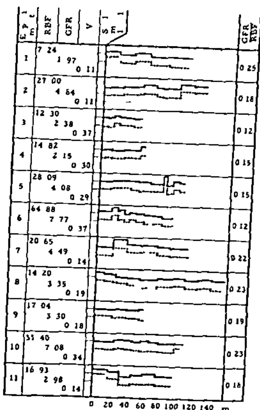


Fig 4 Renal blood flow (RBF) glomerular filtration rate (GFR) and urine flow (V) in the left kidney in 11 experiments The variations are shown in per cent of the mean value for each experiment — stated in the columns at left The graphic level of each mean value in relation to its diagram is shown immediately to the left of the diagram the mean values being presented as 100 per cent levels The diagrams are arranged so that the space between adjacent mean values always represents 100 per cent

The ratios of GFR to RBF are shown at right.

——— RBF
GFR
urine flow

In two rabbits the CO_2 content in the blood and the blood pH were studied during the experiment It was found that metabolic acidosis developed during anesthesia The urine pH usually fell to about 5 during the first 3 hours of anesthesia

Table I Comparisons between left and right kidneys with respect to RBF GFR urine flow and weight

Experiment no	Renal weight (grams)		RBF (ml/min.)			GFR (ml/min.)			Urine flow (ml/min.)		
	Left	Right	Left	Right	Correlation coeff. Left/Right	Left	Right	Correlation coeff Left/Right	Left	Right	Correlation coeff Left/Right
1	7.57	7.28	7.2	8.0	0.97	2.0	2.4	0.94	0.11	0.15	0.88
2	*11.2*	*10.5*	*26.5*	*26.2*	*0.99*	*4.8*	*5.0*	*0.97*	*0.11*	*0.12*	*0.96*
3	15.7	14.1	19.3	21.0	0.94	2.4	3.2	0.92	0.37	0.51	0.93
4	9.33	9.18	14.8	18.3	0.98	2.2	2.7	0.84	0.30	0.37	0.99
5	14.3	14.4	27.5	28.9	0.91	4.0	4.6	0.84	0.29	0.41	0.97
6	15.2	14.1	64.9	63.7	0.95	7.8	8.3	0.99	0.37	0.46	0.99
7	13.8	12.4	20.7	23.9	0.95	4.5	5.6	0.96	0.14	0.19	0.77
8	12.6	11.6	14.2	17.5	0.94	3.4	4.8	0.91	0.19	0.25	0.98
9	*14.0*	*12.9*	*17.0*	*17.6*	*0.79*	*3.3*	*3.3*	*0.96*	*0.18*	*0.18*	*0.98*
10	12.3	12.0	31.4	30.8	0.68	7.1	7.0	0.17	0.34	0.34	0.90
11	10.6	10.6	16.9	19.2	0.94	3.0	3.5	0.98	0.14	0.20	0.95
Mean value for angle coefficient of regression line					*0.94*	—		*0.89*	—		*0.77*

Measurements

Renal extraction of diodrast In all experiments the average renal extraction of diodrast was 80—90 per cent A statistical analysis showed a clear falling tendency in degree of extraction during the experimental period The values are shown in Fig 3 No fluctuations in the extraction fraction were detected In preliminary experiments it was not possible to influence the degree of extraction by infusion of adrenaline (1—20 μg/min) noradrenaline (1—20 μg/min) or pituitrin (1—500 mU) intravenously so long as diuresis continued.

Renal circulation In Fig 4 the flow of blood in the left kidney is illustrated The scale on the ordinate represents the deviation in per cent from the mean value which is stated in the column marked RBF

Strong correlation was found between blood flow variations in the right and the left kidney (Table I) In no case was any difference observed between the kidneys with respect to direction of changes in blood flow On average the right kidney had 20 per cent greater blood flow than the left kidney This difference was significant On the other hand the left kidney was almost invariably the heavier of the two

Glomerular filtration rate In Fig 4 the glomerular filtration rate is expressed in the same units as the blood flow i e in per cent of the mean value The correlation between variations in filtration in the two kidneys was high as a rule (Table I) In exp 10 the variations in glomerular filtration rate did not

exceed the error of measurement Filtration was significantly greater in the right than in the left kidney

Filtration fraction Analysis showed in most experiments strong correlation between variations in blood flow and filtration rate in the left kidney implying that the filtration fraction was constant throughout. The ratio of GFR to RBF (Fig 4) varied widely from experiment to experiment.

Urine flow The urine flow in the left kidney is likewise stated in Fig 4 It is seen that the values often varied independently of RBF and GFR. Distinct mutuality of variation between the right and the left kidney was also present with respect to urine flow (Table I) the right kidney showing significantly higher values

Discussion

The experimental observations indicated that in preparations such as those described, the renal blood flow glomerular filtration rate and urine flow can be kept fairly stable for long periods It would also seem possible to produce variations in all three functions by simple measures such as expanding or diminishing the blood volume or the extracellular fluid space by infusion of loss fluid. In these variations renal blood flow and glomerular filtration rate are correlated. Between the experimental preparations no significant differences with respect to the mentioned properties were observed. Nor need differences between the two kidneys as regards variations in urine flow blood flow and glomerular filtration rate be reckoned with Therefore unless advantageous for other reasons it is superfluous to catheterize the ureters separately Consequently such preparations would seem to be suitable for pharmacologic studies provided that the possible effects of anesthesia on tubular function are taken into consideration.

The strong correlation between renal blood flow and glomerular filtration rate in the individual experiments was in good conformity with observations described by FORSTER (1947 DICKER and HELLER (1945) SCHOU (1942) and LAAKE (1945) These writers found that increased diuresis was accompanied by increasing glomerular filtration and renal blood flow Later studies by BROD and SIROTA (1949) FORSTER (1952) and JOSEPHSON and KALLAS (1953) showed these findings to be attributable to the fact that the kidneys were initially in a state of vasoconstriction and proceeded to vasodilation in response to fluid loading

The relative constancy of the filtration fraction in the separate experiments of the present study suggests that fluctuations in the renal blood flow of the preparations were produced mainly by changes in the resistance in the vessels supplying the glomeruli The striking mutuality of variation between the two kidneys of each preparation with respect to blood flow and glomerular filtration would seem to argue for a central origin of the constrictor impulses, or for humoral regulation

The tests did not reveal much about tubular function in the experimental rabbits Since the quantity of the injected diodrast was far below the rabbits Tm — maximal rate of tubular transfer — for diodrast, only large fluctuations in tubular function could be reflected as changes in the extraction of diodrast

The cause of the trend towards diminution of diodrast extraction registered during the experiments could not be elucidated BERGSTROM *et al* (1959) found a corresponding trend in human subjects They attributed this to the fact that the content of the diodrast in the blood cells rose during the initial hours of a continuous diodrast infusion regulated so as to maintain a constant arterial concentration LAAKE (1945) found no corresponding increase of diodrast in rabbit blood cells In the present experiments too no elevation of diodrast content in blood cells was observed which could explain the fall in diodrast extraction Of other conceivable explanations splitting of I^{131} away from the diodrast molecule could be rejected as no significant rise in the I^{131} content of the thyroid could be demonstrated

Clear differences were found between the left and the right kidney with respect to weight blood flow glomerular filtration rate and urine flow with lower values for the left kidney These discrepancies might conceivably have been due to anatomic differences or to the catheterization of the left renal vein A search of the literature yielded no evidence that such differences between the kidneys exist in normal rabbits Since I found no corresponding weight difference between the kidneys of control rabbits of the same breed and size the observed discrepancies in the experimental animals would seem to have been a product of the experimental conditions It seems unlikely that the lower values in the left kidney were attributable to raised venous pressure produced by the catheter in the renal vein since the catheter occupied only a minor part of the vein s cross area The catheter probably evoked reflex vasoconstriction by irritation of the vessel walls

The administered dose of reserpine (0 1 mg 18—20 hours before the experiment) did not seem completely to block the constrictor action of the adrenergic fibres The values for glomerular filtration rate and renal blood flow were lower than those reported by BROD and SIROTA (1949) FORSTER (1952) and JOSEPHSON and KALLAS (1953) Reduction of the catecholamines in rabbit adrenal medulla by more than 90 per cent and in peripheral ganglia (such as the superior cervical solar and inferior mesenterial) by 20 to 60 per cent following administration of 0 1 mg reserpine per kg of body weight was described by MUSCHOLL and VOGT (1958) About one fourth of that dosage was used in the present experiments The observation in preliminary experiments that infusion of noradrenaline or adrenaline produced persistent oliguria also in reserpine-treated animals was in agreement with the finding by ROSELL (1961) that in cats infusion of adrenaline or noradrenaline after reserpine treatment temporarily restores the vasoconstrictor response to stimulation of sympathetic fibres

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From the Department of Histology University of Gothenburg Sweden

The Effect of Oxygen at High Pressure on the Histology of the Central Nervous System and Sympathetic and Endocrine Cells

By

J E Edstrom and H Rockert

Received 13 December 1961

Abstract

Edstrom J E and H Rockert *The effect of oxygen at high pressure on the histology of the central nervous system and sympathetic and endocrine cells* Acta physiol scand 1962 *55* 255—263 — Rats were exposed to oxygen at 6 atm daily for about 8 weeks in one series and for 4 weeks in a second series The exposure times were so adjusted as to give a low incidence of convulsions and lung symptoms The treated animals did not show any evidence of lung damage at autopsy Slight motor symptoms of a paralytic nature developed in some of the rats of the first series after about 2 weeks The symptoms regressed during the continued treatment and had disappeared after further 5 weeks Histological investigation of the nervous system after 7 to 9 weeks showed *no* degeneration *The rats of the second series exhibited* no neurogenic symptoms The exposures to high oxygen tension were found to have an action on the sympathetic nervous system resulting in an increased size of the nucleolus of neurons of the stellate ganglion and the adrenal medulla In both series there was a decrease in the weight of the thymus but the reduction was significant in the second series only In agreement with earlier investigators a weight increase of the adrenals was found ($P < 0.05$ for the first series) and histologically a thickening of the zonal fasciculata A weight increase of the same statistical significance was obtained also for the thyroid The histology of the pituitary was unchanged Determinations of ribonucleic acid and size of nucleolus in the supraoptic nucleus did not reveal any changes The investigation consequently could not provide any evidence for a direct degenerative action of OHP on the central nervous system The observed changes are characteristic for a stress reaction However the weight increase of the thyroid is not typical for stress and may be an expression of a specific OHP effect

In spite of the dramatic action of oxygen at high pressure (OHP) on the nervous system, it has been difficult usually to observe at autopsy or with histopathological methods any consistent changes in the central nervous system (CNS) of animals exposed to it Indeed it has been suggested that the action of OHP on the CNS may be entirely functional (BEAN 1945 a and b for ref) On the other hand, it has been found by BOHR and BEAN (1942), BEAN and SIEGFRIED (1943) and BEAN and WAPNER (1943) that repeated exposure to OHP, even in the absence of convulsions may produce more or less permanent alterations in the functions of the CNS BEAN (1945 b) has reported changes in the white matter of OHP animals showing motor dysfunction.

Like other kinds of stress exposure to OHP shows a particularly pronounced effect on the sympathetic nervous system (BEAN 1956 JOHNSON and BEAN 1957) Data on the morphological correlates of this action however are entirely lacking

Considering the practical applications involving the use of oxygen at increased pressure the reports of permanent nervous changes appearing in rats even in the absence of convulsions deserve attention and should be studied further It is therefore the aim of the present investigation to study mainly with morphological methods the nervous system of rats after repeated exposure to OHP in which convulsions have been avoided as far as possible In order to obtain a more complete picture of the effects of OHP on the animal body a number of endocrine organs were studied in addition

Experimental

In the first series 19 young male rats (Sprague Dawley) weighing 190—210 g at the start of the experiments were used One animal showed an unusual sensitivity towards OHP and died after convulsive attacks at the beginning of the experiments Data from this animal are not included in the results Of the remaining rats 9 were used as controls and 9 were subjected to OHP treatments The animals were placed 3 at a time in an illuminated pressure chamber having a volume of 35 l and provided with a window for inspection from the outside. The chamber was first washed with pure oxygen for a 5-min period after which the pressure was raised under continuous ventilation over a period of 10 min to a maximum of 6 atm absolute (ATA) This pressure was maintained for 7 to 10 min under ventilation and was reduced gradually over a period of 10 min to the atmospheric pressure

The accumulation of CO_2 was moderate in these experiments Assuming a CO_2 production of 5 ml min per rat and using a chamber ventilation rate of 5 l min the maximum CO_2 pressure in the chamber will be 5 mm Hg (216 ml CO at atmospheric pressure)

The exposure to OHP was selected so as to give a maximal effect in the absence of convulsions but seizures could not be avoided entirely To eliminate the risk of convulsions completely it would have been necessary to reduce either the exposure time or the pressure considerably as shown by the great individual variability in sensitivity towards OHP as well as by the individual rat from one exposure to another The rats were given 2 daily treatments 5 1 2 days a week for 7 to 9 weeks Convulsions appeared with an average frequency of 5 per cent usually during the phase of decompression.

The weights of the animals were controlled with the weight curves for the controls and the treated rats coinciding rather well

In the second series 6 controls and 6 experimental animals were treated in the same way but for a shorter period of time (4 weeks) The average incidence of convulsions was 12 per cent in this series

At the termination of the experiments the animals were killed on the day after the last treatment by decapitation after ether anesthesia The lungs were inspected macroscopically and the following organs weighed pineal gland pituitary adrenals thymus thyroid testes lungs heart liver kidneys and spleen Pieces of the nervous system were fixed in neutral buffered formalin and Carnoy s fixative pieces of adrenals testes and lungs and the thyroid were fixed in formalin and the pituitary in Zenker s fluid Some of the formalin fixed material from the central nervous system and from the adrenals were freeze sectioned the remaining material was paraffin embedded

Freeze-sectioned material was stained with Sudan III in different combinations For the CNS the luxol fast blue B of KLUVER in combination with Sudan III (Mc MANUS and MOWRY 1960) was used for normal and degenerating myelin in addition to luxol fast blue B — periodic acid Schiff — haematoxylin Adrenals were stained with Sudan III — hematoxylin — eosin In the central nervous system transverse sections were taken at various levels of the neuraxis viz the mesencephalon including the overlying part of the cerebral hemispheres the pons and cerebellum and the cervical spinal cord

For paraffin embedded material various methods were employed For the CNS (hypothalamus motor cortex cerebellar cortex and spinal cord) and for the stellate and trigeminal ganglia methylene blue according to PISCHINGER (1926) was used The pituitaries were stained according to PEARSE (1950) and the rest of the material with hematoxylin and eosin

Determinations of nucleolar size was carried out on stellate ganglion cells on chromaffin cells of the adrenal medulla on neurons of the supraoptic nucleus of the hypothalamus and on Purkinje cells of the cerebellum For this purpose nuclei containing single nucleoli were used always Determinations of nuclear size were carried out in the chromaffin cells of the adrenal medulla and in thyroid parenchymatous cells Nucleolar size was determined with the aid of an immersion lens, 100 × N A = 1 25 and a calibrated eye piece 14 × and nuclear size by projecting sections in a camera lucida arrangement at a magnification of 1 350 × and measuring the drawn nuclei

Ribonucleic acid (RNA) was determined for nerve cell bodies in nucleus supraopticus of the hypothalamus using a method of microextractions (EDSTROM 1958)

Results

In 4 animals of the first series slight paralytic symptoms manifested themselves after about 2 weeks the most characteristic trait was a tendency towards dragging the hind legs particularly on a smooth support Other symptoms, indicating CNS damage were absent The observed symptoms were variable in strength from week to week and disappeared entirely after a period of about 5 weeks from all rats During the latter part of these 5 weeks a fifth rat also showed slight paralytic signs of the same nature Hyperexcitable post convulsic states which lasted about a day appeared 3 times In a few cases the OHP treatments resulted in post-convulsic dyspnea When this happened, the treat-

Table I Body and organ weights in OHP treated rats

Mean values ± S E M

	Controls	n	Exposed animals	n	P for real difference
	Series 1 7—9 weeks exposure				
Body weights					
Initial	158 g	9	158 g	9	
Final	359 g	9	357 g	9	
Organ weights					
Pineal gland	1 61 mg	6	1 55 mg	8	
Pituitary	9 3 mg	8	10 0 mg	8	
Adrenals absolute weight	47 1 ± 1 89 mg	9	55 3 ± 2.65 mg	9	< 0 05
Adrenals per 100 g	13 1 ± 0 59 mg	9	15.6 ± 0 85 mg	9	< 0 05
Thyroid absolute weight	41 9 ± 2.29 mg	7	53 4 ± 4 07 mg	7	0 05
Thyroid per 100 g	11.6 ± 0 70 mg	7	14 6 ± 1 09 mg	7	< 0 05
Thymus	529 ± 56 mg	8	478 ± 25 mg	8	
Testes	3 39 g	5	3 43 g	5	
Heart	1 11 ± 0 02 g	7	1 15 ± 0 03 g	8	
Lungs	1 49 ± 0 08 mg	9	1 59 ± 0 08 g	9	
Liver	15 3 g	5	15 5 g	5	
Kidneys	2 77 g	5	2 69 g	5	
Spleen	0 66 g	4	0 67 g	5	
	Series 2 4 weeks exposure				
Body weights					
Initial	215 g	6	216 g	6	
Final	310 g	6	322 g	6	
Organ weights					
Adrenals absolute weight	45 1 ± 3 26 mg	6	55 9 ± 2.62 mg	6	< 0 1
Adrenals per 100 g	14 7 ± 1 01 mg	6	16 8 ± 0 95 mg	6	
Thyroid absolute weight	34 4 ± 2.[illegible]0 mg	6	38.5 ± 1 34 mg	6	
Thyroid per 100 g	11 0 ± 0 77 mg	6	12.2 ± 0 71 mg	6	
Thymus	634 ± 31 mg	6	502 ± 35 mg	6	< 0 005
Heart	1 09 ± 0 03 mg	6	1 08 ± 0 03 g	6	
Lungs	1 77 ± 0 08 mg	6	1 70 ± 0 05 g	6	

ments were interrupted for a day for the affected animal In the second series there were no signs of lasting paralytic or hyperexcitable states

The first series showed no difference in body weight between the control and experimental groups in the second series the experimental animals showed a slight weight increase over that of their controls (Table I) There were no signs indicating that the exposures might interfere with general health

II Nuclear and nucleolar diameter sizes after exposure to OHP (series I) Numbers gi en thesis indicate number of animals and number of nuclei or nu leoli per animal respecti ely values ± S E. M

	Controls μ	Exposed animals μ	Change	P
al nucleoli				
ate ganglion	3 13 ± 0 050 (8 25)	3 40 ± 0 037 (9 25)	+ 8 6	< 0 001
aoptic nucleus	3 24 ± 0 043 (6 25)	3 35 ± 0 037 (6 25)	+ 3 5	Insign
inje cells	3 10 ± 0 027 (6 25)	3 15 ± 0 035 (6 25)	+ 1 5	Ins gn
affin cells of the l medulla				
ei	7 17 ± 0.15 (6 100)	7 53 ± 0 15 (6 100)	+ 5 0	Ins gn
eoli	1 96 ± 0 033 (6 25)	2 08 ± 0 008 (6 25)	+ 6 1	< 0 01

weight recordings of a number of organs are given in Table I It can n that there are only slight and insignificant changes in lung and heart ts as a result of OHP exposure which tends to confirm the macro- as well croscopic findings of the lungs that these were completely free from de ble pathological changes Most other organs as well showed unchanged its In the case of the thymus there was a 10 per cent decrease in weight e first series statistically insignificant but the second series showed a y significant decrease (P < 0 005) as a result of the OHP treatments xplanation for the difference among the two series may be found in the r convulsion frequency in the second series and in the fact that the ani belonging to this series were younger at the time of sacrifice giving rela higher control values The thyroid and the adrenals show increased nts In the first series the P value is less than 0 05 for both organs irrespec- of whether the absolute weights or the weights per unit body weight are The weight of the thyroid showed a smaller insignificant increase in the nd series Material for histological investigation of the thyroid was taken tunately from this series only Nuclear size and the histological picture unchanged in this material The microscopic examination of the adrenals ated an increased hormonal activity The zona fasciculata was thickened comparatively rich in lipids stainable with Sudan III In the adrenal illa measurements of nuclear and nucleolar sizes indicated a hypertrophy aking an increased activity (Table II)

view of earlier reports of an atrophic action of OHP on spermatogenesis RIO DE ALMEIDA 1934) the testes were investigated histologically using the gen reaction No evidence of any impairment of spermatogenesis was found histological investigation of the pituitary did not reveal any changes in roportion between the acidophils basophils and chromophobes as far as l be estimated subjectively

Table I Body and organ weights in OHP treated rats

Mean values ± S E M

	Controls	n	Exposed animals	n	P for real difference
	Series 1 7—9 weeks exposure				
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Initial	198 g	9	198 g	9	
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Organ weights					
Pineal gland	1 61 mg	6	1 75 mg	8	
Pituitary	9 3 mg	8	10 0 mg	8	
Adrenals absolute weight	47 1 ± 1 89 mg	9	55 3 ± 2.65 mg	9	< 0 05
Adrenals per 100 g	13 1 ± 0 59 mg	9	15 6 ± 0 85 mg	9	< 0 05
Thyroid absolute weight	41 9 ± 2.29 mg	7	53 4 ± 4 07 mg	7	< 0 05
Thyroid per 100 g	11 6 ± 0 70 mg	7	14 6 ± 1 09 mg	7	< 0 05
Thymus	529 ± 36 mg	8	478 ± 29 mg	8	
Testes	3 9 g	5	3 43 g	5	
Heart	1 11 ± 0 02 g	7	1 15 ± 0 03 g	8	
Lungs	1 49 ± 0 08 mg	9	1 59 ± 0 08 g	9	
Liver	15 3 g	5	15 5 g	5	
Kidneys	2 77 g	5	2.69 g	5	
Spleen	0 66 g	4	0 67 g	5	
	Series 2 4 weeks exposure				
weights					
Initial	215 g	6	216 g	6	
Final	310 g	6	322 g	6	
Organ weights					
Adrenals absolute weight	45 1 ± 3.86 mg	6	53 9 ± 2.62 mg	6	< 0.1
Adrenals per 100 g	14.7 ± 1 01 mg	6	16.8 ± 0 95 mg	6	
Thyroid absolute weight	34.4 ± 2.20 mg	6	38.5 ± 1 94 mg	6	
Thyroid per 100 g	11 0 ± 0 77 mg	6	12.2 ± 0 71 mg	6	
Thymus	694 ± 31 mg	6	502 ± 35 mg	6	0 005
Heart	1 09 ± 0 03 mg	6	1 08 ± 0 03 g	6	
Lungs	1 /7 ± 0 08 mg	6	1 70 ± 0 05 g	6	

ments were interrupted for a day for the affected animal In the second series there were no signs of lasting paralytic or hyperexcitable states

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tion may occur (BEAN 1945 b) but that rather severe exposures are required (BEAN personal communication) Clearly more work is needed for the elucida tion of this aspect of the action of OHP

It has been found that OHP exerts a stimulating action on the symphathetic nervous system (BEAN 1945 a for ref) where maybe the most striking effect of an increased sympathetic tone is seen on an inhibited gastrointestinal peristalsis (TROELL 1947) The findings of increased nucleolar size in neurons of the stel late ganglion as well as in the homologous chromaffin cells, are thus in agreement with the view that the sympathetic system was stimulated (The grounds for using values on nuclear and nucleolar size as indices of cell activity rest on empirical observations rather than on an understanding of what this hyper trophy represents in chemical terms However there seems to exist a general agreement that increased size is an expression of increased activity both with regard to the endocrines and the nervous system see BENNINGHOF 1950 EICHNER 1953 MILLER 1954 and EDSTROM 1957) It would be of interest of course to determine whether histological signs of increased activity are to be found centrally perhaps at the preganglionic level JOHNSON and BEAN (1957) con cluded, on the basis of pharmacological experiments that OHP stimulated the sympathetic nervous system and that ganglionic blocking agents reduced convulsions as well as pulmonary damage while peripheral sympathicolytic compounds did not protect against the convulsions

It is thus likely that the influence of OHP on the adrenal cortex is mediated largely through the sympathetic nervous system and the adrenal medulla Its increased activity in turn would stimulate the pituitary to an enhanced production of ACTH It is known from earlier workers that adrenal hypertrophy occurs after OHP (BEAN 1951 BEAN and JOHNSON 1954) and this was con firmed in the present investigation Apart from the weight increase one can observe histologically a thickening of the zona fasciculata Whether this zone is depleted of stainable lipids or not will depend upon the length of time that has elapsed between exposure and sacrification In our experiments, where the animals had become adapted to the treatments and were allowed to rest overnight before they were killed a lipid rich zone could be demonstrated In the animal that died after a convulsive attack early during the treatments the zona fasciculata exhibited a low content of stainable lipids It is known that the response of the adrenal resulting from OHP exposure is a part of the general stress adaptation syndrome and it is possible that the stress evoked response renders the animals more sensitive towards the adverse effects of oxygen Adrenalectomy (GERSCHMAN and NADIG 1952) as well as hypophysectomy (BEAN 1952 BEAN and JOHNSON 1952 BEAN and SMITH 1953) are in fact meas ures tending markedly to improve the resistance of rats towards OHP Adrenal ectomy might also owe its therapeutic effects to the elimination of adrenaline which aggravates the symptoms of OHP poisoning even in the absence of the adrenal cortex (BEAN and JOHNSON 1955)

It is evident however, that the effects of hypophysectomy are more complex than that which can be ascribed to the elimination of ACTH only GROSSMAN and PENROD (1949) and SMITH *et al* (1960) found that the thyroid influences the severity of the reactions following exposure to oxygen at increased pressure, thyroxin aggravating and surgical or chemical thyroidectomy improving the viability The recording of a weight increase of the thyroid in one of the series is consequently of some interest If this is a representation of an increase in activity the response of the thyroid to exposure to OHP parallels that of the adrenal, *i e* it reacts in a manner reinforcing the harmful effects of OHP Since the activities of the adrenal cortex and the thyroid are reciprocal (HARRIS 1955 for ref) this would indicate also that the action of OHP is of a specific character and not merely an unspecific stress situation

The present work was carried out with the aid of a grant from the Swedish Navy We would like to thank Dr L TROELL, Surgeon General of the Navy for his interest and support and Professor H HYDÉN for providing facilities to do the experimental work at the Department of Histology University of Gothenburg We are also indebted to Professor J W BEAN Ann Arbor Michigan and Dr E KOENIG for scrutinizing the manuscript and correcting the English text Finally our thanks are due to Miss INGBRITT SANDELL and Miss MARGARETA HEDIN for careful technical assistance

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Acta physiol scand 1962 55 264—269

From the Institute of Physiology University of Uppsala Sweden

Electrolyte Effect of Cardiac Glycosides on Dog's Heart-Lung Preparation

By

N H Areskog

Received 15 December 1961

Abstract

Areskog N H *Electrolyte effect of cardiac glycosides on dog's heart lung preparation* Acta physiol scand 1962 *55* 264—269 — The electrolyte effect of the rapid acting heart glycoside convallatoxin was investigated on heart lung preparations of dog labelled with ^{42}K and ^{24}Na Total concentrations of plasma potassium and sodium were determined by flame photometry The radioactivity of plasma heart lymph and myocardial samples was determined by gamma spectrometry A net loss of potassium from the myocardium was induced by the glycoside but there was not a corresponding loss of ^{42}K This means that isotopic equilibrium was not attained in spite of an equilibration time of 60—90 min and the conclusion is drawn that the glycoside mainly affected a part of the intracellular myocardial potassium which has a relatively slow exchange rate The changes in heart lymph were principally the same as in plasma No significant changes of the sodium balance due to the glycoside action was found

In a previous study (Areskog 1962) the effects of two rapid acting heart glycosides convallatoxin and acetyl strophanthidin were studied on dog's heart lung preparation (HLP) One of the main effects of cardiac glycosides is the influence on the electrolyte balance of different kinds of cells (e g Calhoun and Harrison 1931 Wood and Moe 1942 Schatzmann 1953) This action results in an increase of the potassium loss from the cells In the previous study the electrolyte effect of the cardiac glycosides was studied in relation to the positive inotropic effect and the toxic effect (provoking of arrhythmia) and also the influence of pH

In order to analyze further this electrolyte effect the movements of ^{42}K and ^{24}Na have been studied in the HLP Flame photometry (used for analysis of potassium and sodium concentrations in plasma in the previous paper) has been complemented with isotope technique in order to get a conception not only of net fluxes but also of unidirectional fluxes and exhange rates

Methods

Except for the isotope technique the methods are the same as in the previous paper (ÅRESKOG 1962)

The HLP was labelled with ^{42}K (100—200 μC as KCl) and in most experiments also with ^{24}Na (75—100 μC as NaCl) The total amount of potassium and sodium in the radioactive samples introduced did not measurably change the concentrations in plasma. After an equilibration time of 60—90 min the heart glycoside was injected The glycoside used was convallatoxin (Convalyt Dr Madaus & Co Cologne) in a dose of 0 005 mg/kg body weight of the intact dog The circulating blood volume in the HLP system was about 400 ml and the heart weights about 100 g

A lymph vessel draining mainly the left ventricle was cannulated in some experiments[1] and lymph samples were collected The method is laborious since it is very difficult both to cannulate the lymph vessel and to get a good flow in it because of its very small calibre and the difficulty of identifying it after incision

Measurements of the radioactivity The ^{42}K and ^{24}Na activity in plasma, blood and in some cases myocardial samples from the right auricle were measured by a two channel gamma spectrometer (ÖBRINK and ULFENDAHL 1959) The samples were counted in two fixed energy channels a technique which presupposes a very high stability of the electronic equipment For measurement the plastic sample tubes are enclosed in brass cases to screen the detector from the β radiation (especially the high energy β radiation of ^{42}K)

With a suitable choice of channels the formulas of Öbrink and Ulfendahl for the calculation of the activity can be simplified If only ^{24}Na is counted in the high energy channel (channel I) and the other channel (channel II) which is wide open counts the total activity from both ^{42}K and ^{24}Na the following equations for *calculation of* the activities of ^{42}K and ^{24}Na are obtained

$$C_K II = \frac{1}{k_K II}\left(N_{tot} II - \frac{k_N II - N_{tot} I}{q_N}\right) \tag{1}$$

$$C_K II \; k_K II + C_N II \; k_N II = N_{tot} II \tag{2}$$

$$k II = \exp\left(\frac{-0.69 \; t II}{T 1/2}\right) \tag{3}$$

$$q_N = \frac{C_N I}{C_N II} \text{ (for a standard sample only containing } ^{24}Na\text{)} \tag{4}$$

$C_N I$ $C_N II$ = counts per min of ^{24}Na in channel I and II at time 0
$C_K II$ = counts per min of ^{42}K in channel II at time 0
$k_N II$ $k_K II$ = correction factors for decay between time 0 *and time for actual counting* (t II)
$N_{tot} II$ = total counts per min in channel II at time for actual counting
T 1/2 = half life time for the actual isotope

[1] The author is indebted to Dr C GROTTE for expert assistance in these experiments

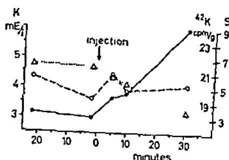


Fig 1

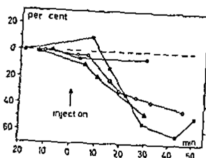


Fig 2

Fig 1 Total potassium concentration (K mE/1 filled circles) ⁴²K concentration (counts per min/g plasma × 10⁻⁴ open circles) and a measure of specific activity of K (S relative units open triangles) in plasma before and after injection of convallatoxin to a HLP

Fig 2 Percental change of the specific activity of K in plasma after injection of convallatoxin Different symbols denote different experiments

As is clear from the equations correction for decay is made This is necessary as the isotopes used have short half lives

Apart from the counting error (S D = $\sqrt{\text{number of counts}}$) it is possible to determine the two isotopes in a ^{42}K ^{24}Na mixture with an accuracy of 1—3 % (ÖBRINK and ULFENDAHL personal communication) if at least 10 000 counts are noted for every sample in the wide open channel

Results

Potassium

The results of the experiments showed constantly a discrepancy between the findings of the flame photometry and the results from isotope analysis with regard to potassium concentration This is illustrated in a typical experiment (Fig 1) After the administration of the glycoside the ^{42}K concentration of plasma increases only slightly and transiently in contrast to the considerable and steady increase of the total potassium in plasma This means that the specific activity of plasma decreases (i e the ratio ^{42}K/total K) The other experiments gave a similar result (Fig 2) Compared to the preinjection values the decrease of specific activity was significant in 11—30 min (P 0 01—0 001) and 31—50 min ($P < 0\,001$) after the injection but was not significant 30—20 min ($P > 0\,1$) before and 1—10 min ($P > 0\,1$) after the injection One might therefore expect the specific activity of myocardium to show a corresponding increase and the analysis of some serial myocardial samples tended to confirm this expectation The concentration of ^{42}K in myocardial samples from the right auricle increased in two out of four experiments (by 9 and 21 per cent respectively at 15—20 min after the injection) and was unchanged in two experiments in spite of the net potassium loss to plasma

The findings illustrate that isotopic equilibrium was not reached within the system during the equilibration time The results suggest that the glycoside

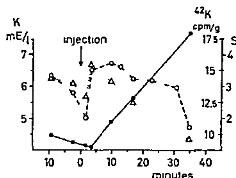


Fig 3 Total potassium concentration (K mE/l filled circles) K concentration (counts per min/g lymph × 10^{-4} open circles) and a measure of specific activity of K (S relative units open triangles) in heart lymph from a heart lung preparation before and after injection of convallatoxin

action mainly affected a compartment of intracellular myocardial potassium which had not reached isotopic equilibrium and had a low specific activity in spite of the rather long equilibration time (60—90 min) *i e* the affected potassium compartment should have a relatively slow exchange rate

Of the lymph experiments only one was entirely satisfactory from a technical point of view and the results from this experiment are illustrated in Fig 3 The changes of labelled and total potassium concentration in heart lymph were principally the same as in plasma after injection of the heart glycoside

Sodium

The plasma concentration of ^{24}Na and total sodium did not change significantly In three out of five experiments the ^{24}Na concentration of plasma tended to change in antiphase to total potassium in plasma but this finding was not judged to be significant (Table I) The average change of total sodium in the percentage of individual initial levels amounted to + 1 4 per cent during the first 10 minute period after the injection and to + 0 7 per cent about 25 min after the injection of the glycoside

Table I Percental change of ^{24}Na concentration in plasma before and after injection of the glycoside in five heart lung preparations n equals number of determinations M is mean percental deviation from "control" value at time of injection and P is probability of significance (t test) that the mean values do not differ from the "control" values

	Before injection min	After injection, min				
	30 — 10	1 — 10	11 — 20	21 — 30	31 — 40	41 — 50
n	7	8	6	5	5	4
M	−1 3	−4 6	+ 1.3	−3 4	−5 4	−1.2
P	> 0 10	0 05 — 0 10	> 0 10	> 0 10	0 05 — 0 10	> 0 10

Discussion

The effect of cardiac glycosides on cation movements has been extensively studied during the last decade. In red cells for example several workers have shown that the glycosides act by blocking the 'active' transport, while the passive fluxes are not affected (e. g. SCHATZMANN 1953, GLYNN 1957). In muscles (e. g. HAJDU 1953) it was shown that the active inward transport of potassium was suppressed by the glycosides.

In the present system there was a net loss of potassium from the myocardium which is in agreement with the majority of earlier reports (e. g. WOOD and MOE 1942, BLACKMON et al. 1960). This finding might well depend on a mechanism which blocks the inward transport of potassium. The basic mechanism and site of such a blocking effect is still of course unknown. As have been pointed out in the previous paper (ARESKOG 1962) it is probable that a part of the potassium increase in plasma was due to the lungs, whereas no measurable influence of the glycoside on the red cells could be detected with the doses used.

In order to explain the behaviour of tracer potassium and ordinary potassium a two-compartment system for the myocardial potassium was proposed in a preliminary note (ARESKOG 1961). The term compartment is not intended necessarily to indicate any anatomically distinct region but merely implies that there are two different rates of potassium exchange. The proposed system would consist of one rapidly exchangeable component and one component with a low exchange rate. In the present experiments it has been shown that isotopic equilibrium with the whole myocardial potassium could not have been attained. In terms of the present model this means that the slow compartment, at least, was far from equilibrium, and it is on this slowly exchangeable phase that the glycoside action is supposed to be mainly directed, perhaps blocking the active inward transport. The concentration in the fast compartment would increase secondarily to a decrease in the slow one. The intracellular labelled potassium will then increase continuously since the system is closed. The present results are explicable in terms of this model. Both the results and the model are in agreement with the results of SCHREIBER (1956) who found that the myocardial potassium of frog heart existed in two phases and that ouabain caused an inhibition of potassium entrance into the slowly exchanging phase, while the fast compartment exchanged freely. SCHREIBER, ORATZ and ROTHSCHILD (1961) have recently confirmed these results on the isolated rabbit heart. In contrast WOOD and CONN (1958) found mainly one exchange rate of ^{42}K on isolated perfused dog hearts. However, their data indicated the presence of an additional intracellular compartment (comprising about 19 per cent of the total) with a slower exchange rate. In their discussion they do not exclude the possibility of an inhomogenous potassium pool including an additional small compartment exchanging at a relatively slow rate.

The investigation was supported by grants from the S edish National Assoc ation against Heart and Chest Diseases

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Acta physiol. scand 1962 55 270—275

From the Geriatric Department Sodersjukhuset Stockholm Sweden

Studies on the Effect of a Nitrate Compound on Arterial Hemodynamics in Human Beings

By

I G Porjé and B Rudewald

Received 18 December 1961

Abstract

Porjé I G and B Rudewald *Studies on the effect of a nitrate compound on arterial hemodynamics in human beings* Acta physiol. scand 1962 55 270—275 — The effect of 1 hydroxy 2 2 bis (hydroxy methyl) butanetrinitrate (Etrynit Bofors) on arterial blood pressure aortic blood flow and left ventricular external work (power) in human beings has been studied with a pressure gradient method An unsteady state is induced with rapid changes which cannot be studied with methods based on observations of mean flow over an appreciable time A transient increase of cardiac output and left ventricular work may be observed during the first 3—4 min and then fluctuating decrease of these modalities may follow The effect of the drug on blood acceleration and arterial blood pressure is discussed

Despite the extensive use of nitro compounds in heart and vascular diseases our knowledge of their effect and mechanism is far from complete

Honig Tenney and Gabel (1961) who in an excellent work studied the effect of nitroglycerine on circulation in dogs consider that the controversial results obtained in the study of the effects produced by this substance are due to the methods applied being based on mean flow determinations Such methods presuppose however a steady state — but actually we are dealing with a rapidly fluctuating unsteady state

According to the classical theory the effect of nitroglycerine depends on vasodilatation of the coronary vessels and an increase in the coronary blood flow According to recent investigators nitroglycerine reduces both cardiac work and the oxygen requirements of the heart muscle Improvement is said to be due to the fact that coronary blood flow is decreased but not to the same extent as cardiac work (Fronek and Ganz 1960) Honig Tenney and Gabel (1961) however found that as aortic pressure fell cardiac output increased from 10 to 55 per cent for 1/2—3 1/2 min then fell 10 to 25 per cent below control levels as the diastolic pressure recovered

Fig 1 Diagram showing the position of the catheter in the aorta

In this connection it may be mentioned that STAM and HONIG (1961) maintain that nitroglycerine changes the metabolism of the heart muscle in such a way, that it 'uncouples' the oxygen consumption from the high energy phosphate bond formation and reduces the chemical efficiency

The differential pressure method described by PORJE and RUDEWALD (1961) has made it possible to study in man the hemodynamic effects of drugs at any given moment of time Here only an outline of the principle involved will be given

Method

Hemodynamic data on the motion of blood in the human ascending aorta can be determined from a measured pressure gradient Such data are instantaneous blood acceleration and velocity and by recording the pressure simultaneously it is possible to determine the external work of the left ventricle from one moment to another In order to measure the pressure and pressure gradient a double lumen catheter is positioned in the ascending aorta (Fig 1) The differential pressure between two points separated by a short distance along the vessel is measured by a special differential pressure transducer For technical details and calculation of data see Porje and Rudewald (1961) and Rudewald (1962)

Results

We have studied by this method the effect of a nitrate compound 1 hydroxy-2 2 bis (hydroxymethyl) butantrinitrate (Etrynit Bofors) on arterial hemodynamics in 7 cases The types of cases

Sex	Age in years	Diagnosis
Man	39	Heart observation
Man	46	Heart observation
Woman	50	Pseudoxanthoma elasticum
Man	[illegible]	Hemiparesis
Woman	39	Pseudoxanthoma elasticum
Woman	64	Orthostatic hypotension

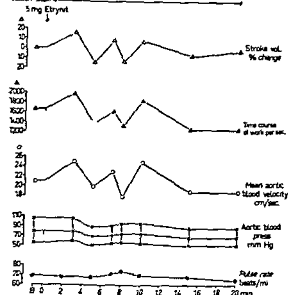


Fig 2 Diagnosis Heart Observation The effect of 5 mg Etrynit in a 46-year-old man with vasolability The examination is carried out in the supine position B on the abscissa indicates basal values The drug is administered sublingually at O and resorbed after 2—3 min Top diagram *shows the percentile change of stroke* volume which is observed to fluctuate during the first 10 min and then *stabilizes on the basic level. A similar behaviour is observed in the time* course of work and then mean aortic blood velocity The mean aortic blood pressure and the pulse amplitude is slightly reduced during the experiment The pulse rate is on the whole unaffected

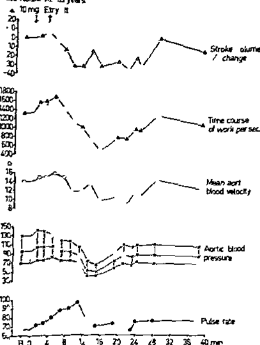
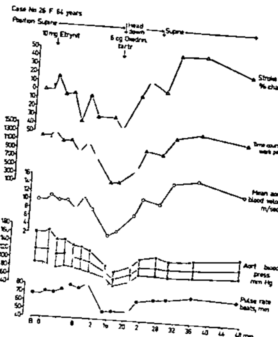
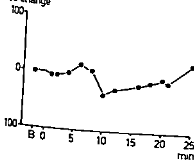


Fig 3 Diagnosis Hemiparesis. The effect of *10 mg Etrynit in a 53-year old man with hemiparesis.* The examination is carried out in the supine position *B on the* abscissa indicates basal values The drug is administered *sublingually at O and resorbed after 5 min (indicated by a dotted line)* Stroke volume and pulse amplitude increase during the first *3—4 min and* then decrease The mean arterial pressure is slightly elevated during the first *10 min of the experiment.*

Fig 4 Diagnosis Orthostatic hypotension The effect of 10 mg Etrynit in a 64-year-old woman with orthostatic hypotension The examination is carried out in the supine position B on the abscissa indicates basal values The drug is administered sublingually at O and absorbed after 3—4 minutes There is a transient increase of the stroke volume and the pulse amplitude during the first few min followed by a decrease in these modalities The mean aortic blood pressure falls and the pulse rate increases After about 10 minutes the pulse rate drops abruptly and the patient feels weak. By lowering the head and injecting 6 cg of Oxedrini tartras the patient recovers rapidly Stroke volume and blood pressure are stabilized after about 20 minutes





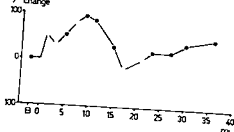


Fig 5

Fig 6

Fig 5 Diagnosis Vasolability Ascending aortic blood acceleration in case no 24 The acceleration increases by about 20 in the early part of the examination and then falls

Fig 6 Diagnosis Orthostatic hypotension Ascending aortic blood acceleration in case no 26 There is a very pronounced gradual increase of the acceleration during the first 10 min of the examination.

The patients were given 5—10 mg Etrynit in tablets which were resorbed in the oral cavity Subsequently series of records of pressure and differential pressure were taken at 2 min interval In order to obtain a steady state in the circulation the patients were allowed to rest for 10 min before the drug was

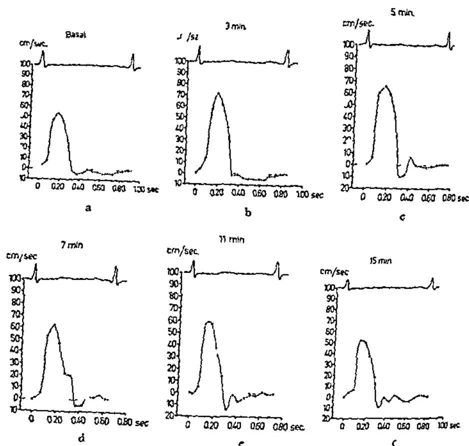


Fig 7 Curves showing instantaneous blood velocity in the ascending aorta before and after 10 mg Etrynit

administered During this period the mean flow varied less than 5 per cent in agreement with earlier results from periods up to 15—30 min

We found that the drug provoked an unsteady state in the circulation as shown by the figures illustrating the effect of the drug This unsteady state was observed in all cases

This finding means that the cardiac output may increase during the first few minutes, and then decreases with certain fluctuations (Fig 2, 3 4 7) At the same time the arterial pressure may initially be unaltered decreased or somewhat increased and then on the whole falls (Fig 2 3 4 7) The left ventricular work may increase somewhat during the first few minutes During the following minutes a marked decrease of the work is observed in these cases

The blood acceleration expressed here as the mean value of instantaneous acceleration during a time interval from the peak of the ECG R wave to maximum blood velocity increased considerably in case no 26 and moderately

in case no 24 during the few minutes In case no 26 (Fig 4) the patient had an episode of weakness and to prevent fainting her head was lowered and oxedrini tartras was given

Discussion

The investigation shows that the nitrate compound used on this material gives rise to an unsteady state in the circulation Since the changes produced are rapid it is impossible to obtain a correct conception of their character by methods which are based on observations on mean flow over an appreciable time as emphasized by Honig *et al* (1961) With our differential pressure method is it possible to follow these changes The hemodynamic effects of Etrynit on human beings agree to a great extent with the effect of nitroglycerine in dogs shown by Honig *et al* (1961)

Of course the results cannot without further experiments be transferred on patients with coronary insufficiency — nor can any direct conclusions be made about the effect of the drug on angina pectoris attacks

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Acta physiol scand 1962 55 276—285

From the Department of Physiology Kungl Veterinarhogskolan Stockholm 51 Sweden

Self-Stimulation in the Goat

By

NILS PERSSON

Received 20 December 1961

Abstract

PERSSON N *Self stimulation in the goat* Acta physiol scand 1962 55 276—285 — A method is described to obtain intracerebral self stimulation in the goat Permanent platinum iridium electrodes were implanted into the hypothalamus The animal could elicit the electrical stimuli by pressing a switch connected to a stimulator that delivered trains of damped square pulses to the electrodes High rates of self stimulation were obtained in the premamillary region of the hypothalamus between columna fornices descendens and tractus mamillothalamicus The behaviour of the goats during self stimulation was observed as well as the different rates and frequencies of self stimulation that they developed at various electrode sites The influence of different strengths of current on the rate of self stimulation was also observed In some parts where high rates of self stimulation were obtained an increase in current strength up to 1 0 mA did not decrease the rate of self stimulation A sudden change to stimuli in rewarding areas that never before had been tested with electric current immediately developed high rates of self stimulation Amphetamine was tested on self stimulating animals The drug increased the animals urge for self stimulation

Self stimulation was first observed to occur in the rat by OLDS and MILNER 1954 Electrodes were implanted at various places in the rat s brain and the animals were tested in Skinner boxes where they had the opportunity to stimulate themselves electrically by pressing a bar The experiments showed that different self-stimulation responses were obtained in the various parts of the brain tested.

Studies of self stimulation have later also been made in the cat by SIDMAN *et al* (1955) ROBERTS (1958) and in the monkey by BURSTEN and DELGADO (1958) BRADY (1958) and LILLY (1958)

Since a technique for implantation of permanent electrodes in the goat s brain was developed in this laboratory (ANDERSSON PERSSON and STROM 1960) it seemed to be of interest to determine if self stimulation could also be obtained in this species The present series of experiments were made in order to develop a technique suitable for self stimulation in goats and also to study some features of self stimulation in this species

Methods

The experiments were carried out on 4 female goats all remaining in a very good condition during the entire experimental period

The technique for implantation of platinum iridium electrodes was earlier described (ANDERSSON *et al* 1960)

All electrodes were placed in the hypothalamus or in its close icinity In 2 of the goats three electrodes were implanted unilaterally and in the other 2 animals 3 elec trodes were first implanted into the left side of the hypothalamus and then another 3 electrodes were placed in the corresponding positions of the right side of the hypotha lamus

The position of the electrodes was determined first b X ray examination of the head of the animal and when the experiments were finished by histological examina tion

Anatomical control When the experiments were finished the animals were sacrificed and their heads were perfused first with Ringer s solution and then with formalin The brains were fixed in the latter and embedded in celloidin Serial transverse sections 50 μ thick were made through that part of the brain which had been the site of the electrodes The sections were made parallel to the electrode tracks and stained with toluidin blue

Technical equipment The roomy stable pen (1 6 1 2 m) that was used for the self stimulation studies had an opening through which the animal could put its neck In the bottom of this opening was a switch connected to the stimulator In a few days the animals learned to use the switch to obtain an electric stimulus This was generated by a damped square pulse constant current stimulator which was set to a frequency of 50 stimuli per sec and a pulse duration of 3 msec Each time the animal pressed the switch the stimulator generated a train of pulses for a time which could be varied between 0 7—22 4 sec The duration of each pulse train was usually set to 3 0 sec Before getting a new train of pulses the animal had to release the switch bar again It was thus impossible for the animal to get a continuous stimulus by leaning against the bar

Recording of self stimulation frequency was made by a Philips recording potentiometer (type PR 2210) connected to a staircase generator generating one step for each stimulus period After 25 steps corresponding to the width of the recording paper the staircase generator voltage returned automatically to zero

Procedure After implantation of the electrodes the animals were not used for any experiments for at least one week During this time they were placed in roomy stable pens of the same size as that used for self stimulation studies During the second week after the implantation of the electrodes the goats were repeatedly placed in the self stimulation pen Here the animals were at first tested on the different electrodes with stimuli elicited by the observer During these preliminary tests no bar switch was present in the pen The behaviour of the goats was noted at different levels of current strength (0 05 up to 1 0 mA

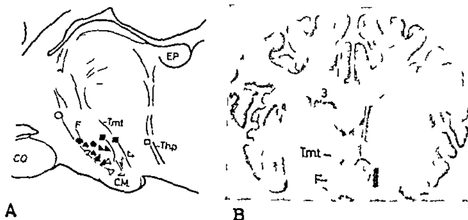


Fig 1 Anatomical localization of the electrode tips
A Sagittal section two millimeters lateral to the midline of the brain Self stimulation rates above 200 per thirty minute periods are indicated by filled symbols and rates below that by open symbols (cf Table I)

B A transverse section along the track of the rostral electrode on the right side in goat II The encircled area indicates the approximate lateral position of all electrodes with self stimulation rates above 200 per thirty minute test (filled symbols in Fig 1 A) Stimulations within the stippled area and more caudally at this lateral level gave no or less obvious responses (goat IV) C M Corpus mamillare C O Chiasma opticum EP Epiphysis F Columna fornices descendens T h p Tractus habenulo-peduncularis T m t Tractus mamillothalamicus 3 V entriculus tertius

When the electrodes had been tested in this way the self stimulation experiments were started The animals were in daily contact with the bar switch in the self stimulation pen Each electrode was tested for half an hour following which the animal was allowed to relax for at least an hour until another electrode was connected Towards the end of the first week it was generally obvious which stimulus strength that had to be used in order to obtain the highest rate of self stimulation at each electrode Thus due to daily tests obvious self stimulation developed within a week and the rate of self stimulation varied with the position of the electrode tips and the strength of the stimulus

Results

A *Anatomical localization*

The electrodes were implanted within the area of the hypothalamus shown in Fig 1 A and B Most of the electrodes had their tips in the region between the mamillothalamic tract and columna fornices descendens where also the highest rates of self stimulation were obtained Three electrodes were implanted in the medial part of the mamillary region of the hypothalamus and these had very low rates of self stimulation (electrodes CL in goat I and CL and CR in goat II — Table I and Fig 1) A similar negative effect was seen at one electrode implanted in the rostral part of the mesencephalon (electrode CR in goat I — open square in Fig 1 A) and at three electrodes located in the medial part of the cerebral peduncle (goat IV) The most rostrally located

Table I Number of self stimulations during thirty minute periods

Goat no	Electrode	Symbol	Stimulus mA	No of tests	Mean value	Standard deviation
I	AL	◣	0 2	10	269 ± 7	22
	BL	◣	0 2	10	247 ± 13	42
	CL	◺	0 2	11	100 ± 22	73
	AR	■	0 2	10	322 ± 12	38
	BR	■	0 2	10	266 ± 10	31
	CR	□	0 2	10	17 ± 7	21
II	AL	◢	0 2	4	223 ± 14	28
	BL	◢	0 2	4	243 ± 15	30
	CL	◿	0.2	4	44 ± 6	12
	AR	▲	0.2	9	275 ± 14	41
	BR	▲	0 2	8	281 ± 18	49
	CR	△	0 2	9	165 ± 13	40
III	A	○	0 3	10	12 ± 35	11
	B	●	0 3	12	294 ± 15	51
	C	●	0 3	25	342 ± 8	41
IV	A	▽	0 7	3	169 ± 31	54
	B	▽	0 7	3	66 ± 26	45
	C	▽	0 7	4	94 ± 32	65

The electrodes are named with letters A corresponds to rostral, B middle and C caudal positions. L and R refer to the position left and right to the midline of the brain The symbols for the electrodes correspond to those in Fig 1 A The current was set at a constant frequency of 50 stimuli per second and a pulse duration of 3 0 msec In each case a 3 0 sec pulse train was delivered when the animal pressed a bar

electrode (electrode A in goat III — open circle in Fig 1 A) had its uninsulated tip just lateral to the paraventricular nucleus At this site self stimulation did not develop

B *General behaviour*

As stated above the animals learned in less than a week how to receive the stimulus and soon obtained stable self stimulation rates Their urge for self stimulation was reflected in their general behaviour during the experiments Thus when high rates of self stimulation were obtained the goats were unaffected by their surroundings and continued stimulating themselves with high intensity for at least an hour gradually self stimulation became more irregular and less frequent At some electrode sites the stimuli seemed to be unpleasant and thus the rate of self stimulation was low (electrode CR in goat I — Table I) and the goats sometimes even behaved as if punished

Goat I showed during the experiments a tendency to lick offered block salt during the stimulus The short lasting trains of stimuli may explain why

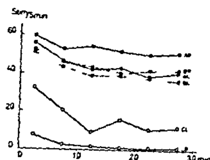


Fig 2 Frequency of self-stimulation in successive five minute periods in goat II during thirty minute tests Stimulus parameters 0 2 mA, 50 sec 3 0 msec 3 0 sec train. For nomenclature of electrodes compare Table I

no alimentary or other conspicuous effects were seen during self stimulation in the other animals Such effects were occasionally seen when longer pulse trains were used

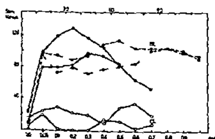
C *Studies on the frequency of self stimulation and the influence of different parameters*

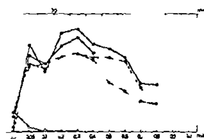
When the animals had reached stable rates of self stimulation they were tested for half an hour on each electrode in successive days to obtain mean values of self stimulation (Table I) No electrode was tested more than once daily and a pause of at least one hour was made until another electrode was tested for self stimulation in the same animal The current was set at the strength that during the previous training seemed to cause the highest rates of self stimulation The animals could start a 3 0 sec train of pulses and then again had to press the bar switch for more stimuli On the basis of stimulation rates the electrodes could be divided into two relatively distinct groups In one group (filled symbols in Fig 1 A and Table I) the rate of self stimulation always exceeded 200 per test period. The mean value for the stimulation rate via the most efficient electrode in this group was 342 stimulations per test period In some experiments rates as high as 400 were obtained.

In the other group the rate of self stimulation was generally much below 200 and the response was irregular (Fig 1 A and Table I — unfilled symbols) All electrodes in goat IV belonged to the latter group (Table I) Obvious self stimulation in this goat was seen to develop only when the stimulus strength was as high as 0 7 mA

Variation in the intensity of self stimulation during single test periods were also studied The recorder made it possible to count the stimuli and analyze the distribution in successive periods The results obtained during such tests in goat I are shown in Fig 2 The results were similar in all the other animals It was thus found that during the first part of the stimulation period the highest rates of self-stimulation were obtained The intensity of self stimulation then decreased slightly throughout the entire period This decline seemed to be independent of the position of the electrode tips

Studies were also made during tests of three hours duration to determine if the decreasing intensity of self stimulation that was observed in the half hour


A


B

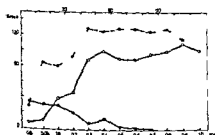

C

Fig 3 Self stimulation in successive ten minute periods at different current strengths Dots and circles indicate values of self stimulation in ten minute periods Fig 3 A and B are values from goat I and Fig 3 C goat III Stimulus parameters 50/sec 3 0 msec 3 0 sec train.

test would continue This happened and the rate of self stimulation was low towards the end of the three hour tests (Fig 5 A)

To determine the variation in frequency of self stimulation at different strength of current tests were made in which the current was increased during successive ten minutes from 0 0 to 1 0 mA as illustrated in Fig 3 A B and C On electrodes with positive response the optimal self stimulation rate was generally obtained when current strength reached 0 2—0 3 mA In some electrodes a further increase in current strength did not affect the rate of self stimulation (Fig 3 A — electrode BL and in Fig 3 C — electrodes B and C) In others a further current increase reduced the self stimulation rate (Fig 3 A — electrode AL Fig 3 B — electrodes AR and BR) In such cases a certain degree of punishment seemed to be mixed with the previously rewarding effect of stimulation and the experiment was not continued up to 1 0 mA

Tests were also made in which the duration of the stimulus train was varied The range tested was between 0 7 and 22 4 sec Generally the rate of self stimulation was inversely proportional to the duration of the stimulus train However at duration below 3 0 sec the animals became restless and extremely eager to press the bar as if the stimulus delivered was inadequate to satisfy their urge for stimulation On the other hand when the duration of stimulus train was 3 0 sec the animals remained relatively calm and the rate of self stimulation was high and with a stable frequency Thus for the further experiments a 3 0 sec duration of the pulse train was used

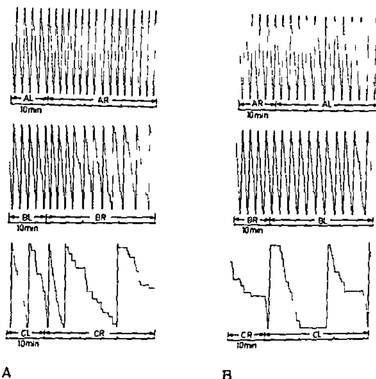


Fig 4 A Recording from goat I indicating a sudden switch of stimuli from electrodes on the left to the right previously untested side Stimulus periods are marked by arrows Every peak represents 25 self stimulations Electrical parameters the same as in Fig 2

B Recording from the same goat the following day showing the similarity of self stimulation rates between the left and right virgin side Electrical parameters the same as in Fig 2

D *Effects of a sudden change of stimulus to the contralateral untested side of the hypothalamus*

In goat I and II three electrodes were implanted on the left side and three others in the corresponding position of the right side of the hypothalamus During the first weeks of self stimulation experiments the response to electrical stimulation was carefully studied on one side only (the left in goat I and the right in goat II) During this time the electrodes of the contralateral side were not used for any electrical stimulation After these two weeks the rates of self stimulation on each of the tested electrodes had become stable Then on the fifteenth day after the beginning of the experiment the stimulus was suddenly changed to the virgin untested side In Fig 4 A are presented the records from the experiment in goat I The intensity of self stimulation at the "virgin side was of the same degree as on the previously tested side The gradual fall in the intensity of the new side was similar to that observed during prolonged stimulation at any effective electrode (Fig 2)

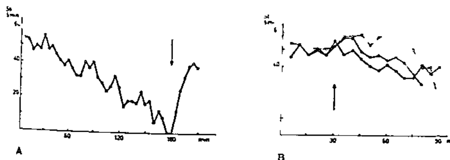


Fig 5 A. Decrease in rate of self stimulation in a 3 hour test and the effect of amphetamine (caudal electrode in goat III) Arrow indicates injection of amphetamine (Phenopromine ACO 1 mg/kg b wt.) Stimulus parameters 0 3 mA 50/sec 3 0 msec 3 0 sec train
B. Self-stimulation during 90 min in goat III Amphetamine was injected after 30 min (arrow) The dose amphetamine and the electrical parameters are the same as in Fig 5 A. Stippled line indicates the rate of self stimulation when the injection was made The unstippled lines show the result of tests with no injection of amphetamine

A corresponding experiment was made the next day This time the "virgin" side was first tested for ten minutes and then the contralateral for half an hour Even in this case the rates of self stimulation on the "virgin" side were of the same intensity as those on the "old" side (Fig 4 B)

The intensity of self stimulation on each "virgin" electrode was then tested in the usual manner with thirty minute tests A comparison between right and left side in goat I and II in Table I shows the results of this test. The deviation that was seen between the rate of self stimulation especially on the posterior electrodes in goat I and II may be due to the fact that their position was not identical bilaterally (Fig 1 A)

E *Effect of amphetamine on self stimulation*

Some experiments with amphetamine were made to study the effect of the drug on the intensity of self stimulation Fig 5 A shows the results of such an experiment In this case a three hour self stimulation period preceded the administration of the drug During this period the rate of self stimulation gradually dropped to zero Amphetamine (Phenopromine ACO 1 mg/kg b wt) given intravenously now almost instantly caused a conspicuous increase of the rate of self stimulation The effect of amphetamine was also tested in another way In this case the drug was injected intravenously in the same amount as in the previous experiment but after a self stimulation period of half an hour only The rate of self stimulation after the injection was markedly higher than that received during a normal period without amphetamine injection One hour after the intravenous injection of amphetamine the rate of self stimulation had returned to the control level (Fig 5 B)

Discussion

The experiments reported here prove that it is possible to obtain self stimulation in the goat The part of the goat s brain that was chosen for these studies was the hypothalamus In the rat, the animal in which self stimulation first was described (OLDS and MILNER 1954) a topographic localization of the various self stimulation effects has been made (OLDS TRAVIS and SCHWING 1960) It was claimed that the structures that yielded behavioural reward upon self stimulation in the rat were a dorsal system in the caudate septal area, the dorsal thalamus and the tectum, and a ventral system mainly located in the medial forebrain bundle These two systems were connected in the region of the anterior commissure The electrodes that were used in the present experiments in goats were according to this nomenclature implanted in the ventral system High rates of self stimulation were found when the electrode tips were located between the mamillothalamic tract and columna fornices descendens in the premamillary region of the hypothalamus (Fig 1 A and B) The proximity to the medial forebrain bundle makes it possible that the current invaded areas of this fiber system However it can not be excluded that the stimulation of other hypothalamic structures may have been involved in the self stimulation response

The study of the influence of different strength of current on the rate of self stimulation also indicates the importance of the position of the electrodes In the present material (Fig 3 A B and C) nine electrodes were tested, three of these produced only slight self stimulation but the other six reached high rates as the current was increased to 0 2—0 3 mA The further increase current strength divided the effective electrodes in two groups In three of the electrodes the rate of self stimulation was decreasing and in the other three the rate was stable as the current was raised The electrode tips were in the first group located in areas with high self stimulation effects but as the current was increased surrounding areas were apparently stimulated causing a disorganization that lowered the rate of self stimulation (Fig 3 A — electrode AL and Fig 3 B — electrodes AR and BR) In cases where the rate of self stimulation was unaffected by the increase in current areas were involved which seemingly were not surrounded by loci with complicating (punishing) effects Thus stable and high rates (Fig 3 A — electrode BL and Fig 3 C — electrodes B and C) of self stimulation were maintained The electrodes that produced a constant low effect of self stimulation as the current was raised might have been placed in neutral areas Furthermore if the rate of self stimulation was immediately decreased with increased current the electrodes may have been located in an area that was negatively rewarding This was especially obvious in the right caudal electrode in goat I located outside the hypothalamus in the anterior mesencephalon (Fig 1 A — open square)

When the goats were observed in self stimulation tests they developed high rates of self stimulation in the beginning of the period, but the rate was falling

as time passed One possibility for this fall is that the animals became satiated by the electrical stimulation This fits drive reduction theories in which satiation occurs as a result of obtaining a reward (OLDS 1958)

It was of interest to study the effect of a central nervous stimulant such as amphetamine on self stimulating animals in respect to this satiety Since the rate of self-stimulation was increased after the administration of the drug it is possible that the threshold for satiation was raised by amphetamine

In the experiments where a sudden change of current was made to a virgin side of the hypothalamus an immediate high rate of self stimulation was obtained This seems to indicate that if self stimulation once has been built up on the basis of stimulus of one particular part of the rewarding system any other part of that system will immediately respond with a high rate of self stimulation

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Acta physiol scand. 1962 55 286—290

From the Institute of Physiology, University of Uppsala Uppsala Sweden

An Automatic Bubble Flow Meter

By

HANS R ULFENDAHL and INGEMAR VOGELER

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Abstract

ULFENDAHL, H R and I VOGELER, *An automatic bubble flow meter* Acta physiol scand 1962 *55* 286—290 — The construction of an automatic bubble flow meter for measurement of arterial or venous blood flow is described The blood passes through a plastic tubing kept at a constant temperature by a water thermostat An air bubble is automatically injected into the tubing and the passage of the bubble is detected by two phototransistors The phototransistor circuit is favourable because of low sensitivity of external pickup The blood flow is correlated to either the number of bubbles per minute or to the reciprocal value of the passage time of one bubble. In both cases a good linear relationship exists between a flow of 15 and 300 ml per minute The flow resistance is 0 03 mm Hg/ml/min

The bubble flow meter is worthy of its good reputation for giving values of high reproducibility (JANSSEN 1957) and thus several constructions are found in the literature (SOSKIN *et al* 1934 DUMKE and SCHMIDT 1942 SELKURT 1949 BAUMGARTNER *et al* 1955 NASH and MILLIGAN 1959, ROCKEMANN 1961)

This paper describes an automatic bubble flow meter with phototransistors as detectors for the air bubble

Description

The flow meter is built on a Perspex plate as illustrated in Fig 1 The blood has to pass through polyvinyl tubing with an inside diameter of 4 mm The tubing is kept at a constant temperature by circulating water from a thermostat The operating solenoid (8 in Fig 1 and S 2 in Fig 2) compresses a latex tube and an air bubble is injected into the streaming blood through a valve (5) On decompression of the latex tube air is sucked through another valve (6) from the air bubble trap (7)

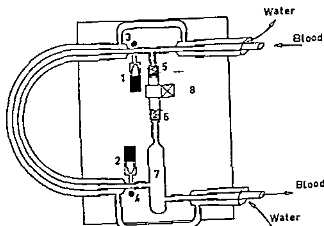


Fig 1 Design of the bubble flow meter
1 and 2 miniature lamps with built in lenses 3 and 4 phototransistors 5 and 6 valves, 7 air trap 8 solenoid

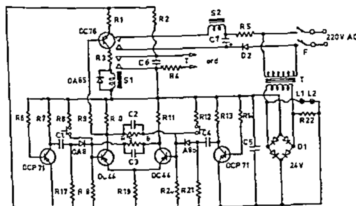


Fig 2 Diagram of the electronic part of the flow meter

R 1 = 150 1 W
R 2 = 100 k
R 3 = 00 1 W
R 4 = 1 M
R 5 = 4 k 3 W
R 6 = 1 M
R 7 = 4 7 k
R 8 = 30 k
R 9 = 10 k
R 10 = 4 7 k
R 11 = 4 7 k
R 12 = 30 k

R 13 = 4 7 k
R 14 = 1 M
R 15 = 20 k
R 16 = 20 k
R 17 = 30 k
R 18 = 2 8 k
R 19 = 510
R 20 = 2 8 k
R 21 = 30 k
R 22 = 100 10 W

C 1 = 50 μF 25 V
C 2 = 470 pF
C 3 = 470 pF
C 4 = 50 μF 25 V
C 5 = 1 000 μF 50 V
C 6 = 50 μF 50 V
C 7 = 32 μF 350 V

S 1 = Relay 24 V 0 5 W
S 2 = Solenoid

L 1 = Philips 2 2 V (1802)
L 2 = do

Tr = 220 V 18 V

During its passage from the injection point to the bubble trap the bubble is registered by two photodetectors 25 cm apart

The detectors consist of two phototransistors (OCP 71) which feed a bistable flip flop circuit (two OC 44) through capacitors (Fig 2) The flip flop circuit is switched on when the air bubble passes the first phototransistor (3) and is switched back when the bubble passes the second phototransistor (4)

The two switches in series with the resistors R 8 and R 12 respectively make it possible to switch the flip flop circuit by hand and thus start and stop the function of the flow meter To the flip flop circuit a switch transistor (OC 76) is connected to operate a relay (S 1) for a timing circuit When the bubble passes the last detector the air pump injects a new air bubble Illumination for the phototransistors is obtained from two miniature lamps (L 1 and L 2) with built in lenses (Philips 2 2 V, 0 18 A) Only simple precautions need to be taken to screen the phototransistors from the light of the room In an unscreened test apparatus a 60 Watt tungsten bulb had to be as near as 30 cm before any disturbance occurred in the proper function of the circuit With the time constant used for the capacitive coupling even the slowest bubble switches the circuit The phototransistor with its low circuit resistances and output impedance has an advantage over photoelements and phototubes in that it is less sensitive to external pickup

The flow resistance is 0 03 mm Hg/ml/min

Recording

The recording of the blood flow through the flow meter can be made in several ways

1 The simplest method is to lead impulses from either of the collectors of the two OC 44 through a capacitor to an ECG apparatus or similar recorder A spike is seen on the kymogram when the air bubble passes the first photodetector and a second inversed spike appears when the second detector is passed The time interval between the two spikes is measured and flow is calculated from a calibration curve (BAUMGARTNER et al 1955)

The relation between the flow and the passage time of one bubble is

$$F = \frac{V}{t} \qquad (1)$$

where

F = blood flow ml per second

V = volume of blood between the two detector points ml

t = time for one bubble to pass between the two detector points, seconds

The equation (1) gives after logarithmic transformation

$$\log F = \log V - \log t \qquad (2)$$

representing a straight line with the slope -1

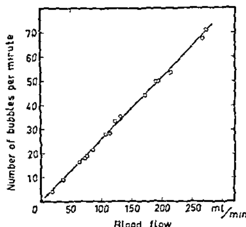


Fig 3 Calibration curve for the bubble flow meter The number of bubbles is counted on a photokymogram The blood flow is measured with a graduated cylinder and a stop watch.

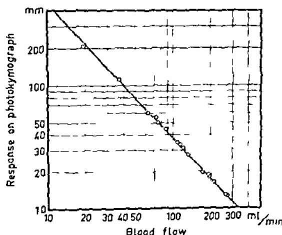


Fig 4 Calibration curve for the bubble flow meter The same experiment series as in Fig 3 Blood flow is correlated to passage time of one bubble (as mm response on the photokymogram)

Thus equation (1) gives a straight line on log log paper

2 A similar method was used by NASH and MILLIGAN (1959) but they measured the time interval between the injection and the arrival of the bubble to a photodetector The accuracy of this method may be influenced by the change in bubble size at different arterial pressures

3 NASH and MILLIGAN (1959) also calculated the flow from the number of bubbles They recorded the blood pressure in the flow meter system and at the injection of every air bubble a small pressure impulse was seen on the record.

We registered the number of bubbles and plotted the result against the flow as seen in Fig 3

The accuracy of the method is good and allows the connection to a tachometer The time constant of the tachometer, however must be high and the reaction to rapid changes in flow is slow

4 BAUMGARTNER *et al* (1955) let the first bubble start an ordinate recorder and the second bubble reset it With this method a reciprocal relation is obtained between flow and response Despite this reciprocal relation this method is used in the present flow meter because of its simplicity When there is no bubble between the two detectors the relay contacts short circuit the capacitor (C 6) in the integrating or timing circuit When a bubble arrives at the first detector the charging of the capacitor begins and continues until the bubble reaches the second detector when the capacitor is discharged The potential over the capacitor is measured with a highly sensitive mirror galvanometer with a large resistance in series The time constant is chosen so that only the first (approximate) linear part of the charge curve is used A calibration curve is seen in Fig 4 The slope of the curve deviates from that expected (cf equation (2)) probably due to the small difference in speed between the blood and the bubble (cf BAUMGARTNER *et al* 1955)

The building of the apparatus was supported by a grant from the Swedish National Association against Heart and Chest Diseases

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Acta physiol scand. 1962 55 291—302

From the Department of Physiological Chemistry University of Lund Lund Sweden

Plasma and Liver Lipids of Ethionine-Treated Rats

By

Thomas Olivecrona

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Abstract

Olivecrona, T *Plasma and liver lipids of ethionine treated rats* Acta physiol scand 1962 *55* 291—302 — The changes in liver and plasma lipid concentrations caused by ethionine have been studied in the rat A dose response curve was obtained for one time interval For a constant dosage the time response curve was measured The response of rats of different sex and nutritional state was also studied Ethionine caused a rise in liver lipids essentially confined to the glyceride fraction a fall of plasma phospholipids cholesterol esters, free cholesterol and glycerides and a rise of plasma free fatty acids (FFA) The liver lipids rose to a higher level in fasted female than in fasted male rats Male rats also required a higher dose of ethionine for maximal response. Carbohydrate feeding to otherwise fasted female rats diminished but did not abolish the liver fat accumulation The changes in plasma and liver lipid levels were maximal 24 hours after the ethionine administration At 72 hours the liver lipids had fallen again almost to the zero time value During the recovery phase the plasma glycerides rose above the zero time value and at 72 hours were 3 times higher

Ethionine the ethyl analogue of methionine inhibits some of the known biochemical reactions of methionine and interferes with hepatic protein synthesis (Farber 1959) It has also been shown to cause a rapid increase in liver lipids (Farber *et al* 1950 Jensen *et al* 1951) and a fall in plasma phospholipids cholesterol and glycerides (Feinberg *et al* 1954 Furman *et al* 1957) This study has been undertaken to obtain detailed information on the effects of ethionine on liver and plasma lipid concentrations

While this study was in progress a report partly dealing with similar experiments was published by Harris and Robinson (1961)

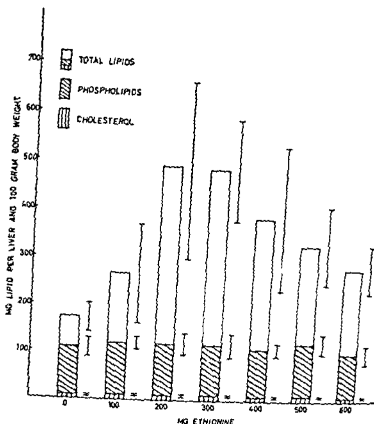


Fig 1 Effect of different doses of ethionine on liver lipids Female rats were fasted for 15 hours then given a single intraperitoneal injections of ethionine and killed 24 hours later Values shown are means of 4 or more rats ± standard deviation of the samples The clear area is the difference between the sum of chemically determined phospholipids and cholesterol and gravimetrically determined total lipids and represents essentially triglyceride

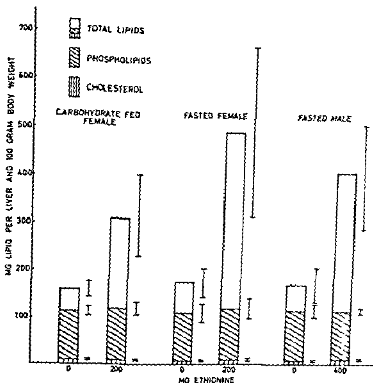
Experimental

The experimental animals were Sprague Dawley rats (obtained from AB Anticimex, Stockholm Sweden reared on a standard pellet diet They weighed 160—190 g at the time of the experiment

Fifteen hours before the injection of ethionine all food was withdrawn and the rats given only water to drink during the experimental time Carbohydrate fed rats were treated similarly but given 20 per cent glucose in half strength saline to drink instead of water

d l ethionine (F Hoffmann — La Roche Basel) was given as a single intraperitoneal injection of a 2 5 % solution in half strength saline Controls were given a similar injection of saline

Rats were killed by exsanguination from the abdominal aorta Clotting of the blood was prevented by the addition of a small amount of the disodium salt of EDTA (Ethylenedinitrilo-tetraacetic acid) Plasma and liver lipid extracts were prepared as previously described (OLIVECRONA 1962 a) Total liver lipids were determined by weight Cnol



3 Effect of sex and nutrition on the response of liver lipids to ethionine Rats were fasted 15 hours then given single intraperitoneal injections of ethionine and killed 24 hours later Carbohydrate fed rats were treated similarly but given 20 % glucose in half strength saline to instead of water Values shown are means of 4 or more rats ± standard deviation of the samples

Results

Effect of different doses of ethionine

Female rats were fasted for 15 hours then given single intraperitoneal injections of ethionine and killed 24 hours later Fig 1 shows the liver lipid levels from this experiment Total liver lipids showed their highest levels at doses of 200 and 300 mg of ethionine Liver phospholipids and cholesterol were within or near normal ranges at all doses of ethionine The rise in total liver lipids thus was due to an increase in liver glycerides JENSEN *et al* (1951) and HARRIS and ROBINSON (1961) have also reported that the rise in liver lipids after ethionine treatment is confined essentially to the liver triglyceride fraction

Fig 2 shows the plasma lipid levels from the same experiment Plasma phospholipids cholesterol esters and free cholesterol were decreased at all doses of ethionine Most of the decrease had occurred by a dose of 100 mg of ethionine Plasma glycerides were decreased at doses of 200 mg or more of ethionine The fall of plasma esterified fatty acids after ethionine administration has been reported previously by FEINBERG *et al* (1954) in the dog, FARMAN

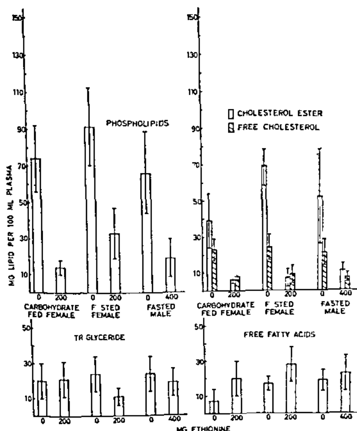


Fig 4 Plasma lipids from the same experiment as Fig 3

et al (1957) in the canine and by HARRIS and ROBINSON (1961) and OLIVECRONA (1962 b) in the rat Plasma FFA were increased at doses of 200 mg or more of ethionine That plasma FFA increase after ethionine treatment has previously been reported by HARRIS and ROBINSON (1961) and by OLIVECRONA (1962 b)

Effect of sex and nutritional state

Female or male rats were fasted for 15 hours then given single intraperitoneal injections of ethionine and killed 24 hours later The carbohydrate fed rats were treated similarly but given 20 % glucose in half strength saline to drink instead of water The female rats were given 200 mg of ethionine as this had previously been shown to give a maximal increase in liver lipids Preliminary experiments on male rats showed that a dose of 400 mg of ethionine gave maximal increase in liver lipids Therefore this dose was given to the male rats

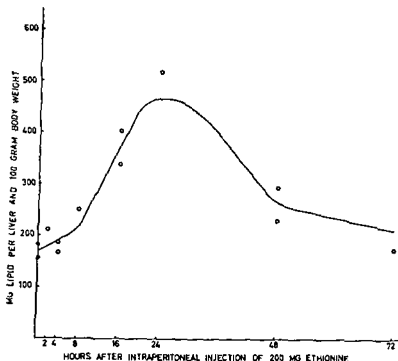


Fig 5 Liver lipid levels at different times after ethionine administration Female rats were fasted for 15 hours then given single intraperitoneal injections of 200 mg ethionine and killed at various time intervals Each point represents values from 1 rat To ease comparison of the data a curve has been fitted visually

Fig 3 shows the liver lipid levels from this experiment Carbohydrate feeding in otherwise fasted female rats diminished but did not abolish the liver fat accumulation FARBER *et al* (1950) reported that carbohydrate feeding prevented the rise in liver lipids after ethionine treatment Their rats were given carbohydrate by stomach tube simultaneously with the ethionine and were killed only 12 hours later Our rats on the other hand drank 20 % glucose in half strength saline ad libitum and were killed 24 hours after the ethionine administration These differences in experimental conditions as well as differences in the strain of rats used may explain the different results

The male rats accumulated less fat in their livers than the females This is in agreement with the results of HARRIS and ROBINSON (1961) but differs from those of JENSEN *et al* (1951) and FARBER *et al* (1951) who reported that male rats accumulated little or no fat in their livers when treated with ethionine

In all series the liver phospholipids and cholesterol were within or near normal ranges so that the increases in total liver lipids were due to increases in liver glycerides

Fig 4 shows the plasma lipid levels from the same experiment All 3 groups showed a decrease of plasma phospholipids cholesterol esters and free cho-

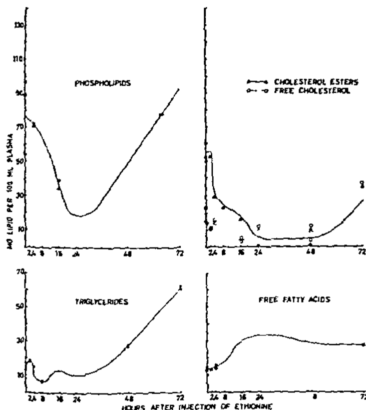


Fig. 6 Plasma lipids from the same experiment as Fig 5

lesterol The fasted females and the fasted males showed a decrease of plasma glycerides whereas these were essentially unchanged in the carbohydrate fed females All three groups showed an increase of plasma FFA. These results are in agreement with those of HARRIS and ROBINSON (1961)

Time response

Female rats were fasted for 15 hours then given single intraperitoneal injections of 200 mg of ethionine and killed at various time intervals

Fig 5 shows the liver lipid levels from this experiment The total liver lipids increased only slightly during the first 8 hours then rapidly increased to a maximum at 24 hours after which they fell off again and at 72 hours were near the zero time value At all times liver phospholipids and cholesterol were within or near normal ranges so that the changes in total liver lipids were essentially changes in liver glycerides

Fig 6 shows the plasma lipid levels from the same experiment Plasma phospholipids fell to a minimum at 24 hours then rose again and at 72 hours

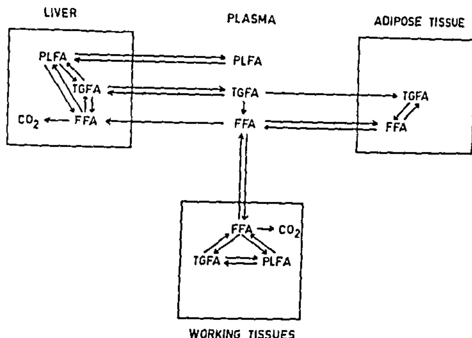


Fig 7 A tentative model for the transport and metabolism of endogenous fatty acids in the rat

showed a mean value similar to the zero time value Plasma cholesterol esters and free cholesterol showed a similar response However the studies were not sufficiently extensive to allow exact definition of the curves Plasma glyc erides showed a doubtful decrease between 4 and 24 hours However the zero time value in this experiment was very low From 24 to 72 hours the plasma glycerides rose rapidly to values 3 times higher than the zero time value The plasma FFA rose and seemed to pass a maximum at 24 hours However, the studies were not sufficiently extensive to allow exact definition of the curve

Discussion

Fig 7 shows a tentative model for the transport and metabolism of endog enous fatty acids in the rat mainly based on data from tracer experiments (OLIVECRONA 1962 a) During starvation plasma FFA are derived mainly from adipose tissue (FREDRICKSON and GORDON 1958) Plasma FFA are taken up by the peripheral tissues and by the liver In either case they are either oxidized or incorporated into tissue fatty acid esters The fatty acid esters in the liver can be incorporated into lipoproteins and as such transferred to the plasma Fatty acid esters from other tissues probably cannot be transferred to the plasma (BYERS and FRIEDMAN 1960 BORGSTRÖM and OLIVECRONA 1961) Plasma triglyceride fatty acids (TGFA) can be taken up intact by the liver (STEIN

and SHAPIRO 1959) and probably also by adipose tissue (RODBELL 1960). It seems likely that they may also be hydrolyzed intravascularly and the liberated fatty acids metabolized as plasma FFA (LAURELL 1959). Plasma phospholipid fatty acids (PLFA) originate in the liver (GOLDMAN *et al.* 1950) and are taken up mainly by the liver (ZILVERSMIT *et al.* 1942).

We have previously shown that the livers of ethionine treated fasted female rats take up and esterify plasma FFA at a rate similar to normal rats but that the further metabolism of liver triglycerides is slower than normal (OLIVECRONA 1962 b). If liver triglycerides are synthesized at a normal rate from plasma FFA but are relatively unavailable for further metabolism, it is apparent that the liver triglyceride pool must increase enormously. According to this hypothesis the liver triglyceride pool remains constant in normal rats because of equilibrium in the rates of FFA esterification into triglycerides and subsequent metabolism of the triglycerides either by transfer to the plasma or by intrahepatic hydrolysis and subsequent oxidation or incorporation into other metabolic pools.

In our previous study of plasma FFA metabolism in ethionine treated rats (OLIVECRONA 1962b) we showed that the fractional turnover rates of plasma FFA in normal and ethionine treated[1] fasted female rats were 1.13 and 0.75 minute^{-1} respectively and that approximately 30 % of the plasma FFA flux was taken up by the liver. Assuming that these figures are the same in this experiment and that the plasma volumes of these rats were approximately 4.0 ml per 100 gram body weight the amount of plasma FFA taken up by the liver per 24 hours can be calculated as follows:

Fasted female rats receiving no ethionine

$1.13 \times 0.17 \times 4.0 \times 0.30 \times 1\,440 = 332$ mg/24 hours and 100 g body weight [2]

Fasted female rats receiving 200 mg ethionine

$0.75 \times 0.28 \times 4.0 \times 0.30 \times 1\,440 = 363$ mg/24 hours and 100 g body weight [2]

The figures are similar in the two groups indicating that the uptake of plasma FFA by the liver was only slightly or not at all increased in the ethionine treated rats in spite of their increased concentrations of plasma FFA. Thus it is doubtful if increased mobilisation of FFA from the depots to the liver is a factor in the pathogenesis of ethionine induced fatty liver.

At doses of 200 or 300 mg of ethionine the fasted female rats showed maximal increase in liver lipids. This amounted on a mean to 315 mg lipid/100 g body weight. If this is all triglyceride it equals about 300 mg fatty acid (calculated as oleic acid). This figure is only slightly lower than the calculated uptake of plasma FFA by the liver per 24 hours and 100 g body weight indicating that in the rats receiving 200 or 300 mg ethionine almost all fatty acids taken up

[1] These rats received 200 mg ethionine divided into 4 equal doses at 2.5 hours intervals and were killed 24 hours after receiving the first dose.

[2] Fractional turnover rate (minutes^{-1}) × plasma FFA concentration (mg/ml) × plasma volume (ml/100 gram body weight) × liver uptake (fraction of plasma FFA flux) × time (minutes).

by the liver remained there and only a small fraction was further metabolized. The plasma FFA, probably, is the only important source of liver fatty acids in fasted rats Thus the amount of fat accumulated in the livers of the rats receiving 200 or 300 mg ethionine was close to the maximal possible amount if as has been indicated above the transport of fatty acids to the liver does not increase appreciably after ethionine treatment It might be predicted that at doses of more than 300 mg ethionine the liver fat accumulation should reach the same or a higher level as at 200 or 300 mg ethionine However rats receiving more than 300 mg ethionine appeared very sick and probably many other factors influenced the results, such as altered cardiovascular dynamics

ROBINSON and HARRIS (1961) have shown that ethionine administration markedly reduces the incorporation of C^{14} leucine into serum lipoproteins and have suggested that a reduction of the rate of synthesis of the protein moieties of the serum lipoproteins occurs at an early stage after the administration of ethionine to the rat They suggested that this is the cause of the depressed serum lipid levels of ethionine treated rats Since all the classes of lipids carried by the lipoproteins (i e phospholipids cholesterol esters free cholesterol and glycerides) were depressed in our rats our results are consistent with this view However the different classes of lipids were depressed to different degrees indicating that there must be qualitative alterations in the plasma lipoprotein composition In recent work from this laboratory ARVIDSON and OLIVECRONA (1962) have been able to show that the fatty acid composition of plasma phospholipids cholesterol esters and glycerides are also changed by ethionine treatment

It has been suggested that decreased transport of fatty acids from the liver as triglycerides is an important factor in the pathogenesis of ethionine induced fatty livers (HARRIS and ROBINSON 1961, ROBINSON and HARRIS 1961, OLIVECRONA 1962 b) In the present experiments the plasma glycerides were depressed in all rats developing fatty livers except the fasted female rats receiving only 100 mg ethionine and the carbohydrate fed female rats However the changes were fairly small and no direct relation between the plasma glyceride levels and the amount of fat accumulating in the liver of the ethionine treated rats could be found An estimation of the importance of the depressed transport of fatty acids from the liver as plasma glyceride would require knowledge of the fractional turnover rate of these in addition to knowledge of their concentrations The possibility exists that the fractional turnover rate of plasma glycerides other than chylomicron glycerides is lower in ethionine treated rats than in normal This has been shown by HARRIS and ROBINSON (1961) and by BORGSTRÖM et al (1962) to be the case for chylomicron glycerides

There was a lag time after the injection of ethionine before the liver lipids began to rise and the plasma lipids to fall This is consistent with the view that the alterations in lipid metabolism were secondary to disturbances of protein metabolism (ROBINSON and HARRIS 1961) The changes in liver and

plasma lipid levels seemed to be maximal at about 24 hours The effects of ethionine then diminished The simplest explanation of this is that at 24 hours most of the ethionine had been metabolized At 72 hours the liver lipids were only slightly higher than the zero time value During the recovery phase the plasma glyceride level rose above the zero time value and at 72 hours was 3 times this value This may represent an intense transport of glycerides from the liver

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From the Department of Physiological Chemistry University of Lund Lund Sweden

Fatty Acid Composition of Plasma and Tissue Lipids of Normal and Ethionine-Treated Rats

By

GOSTA ARVIDSON and THOMAS OLIVECRONA

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Abstract

ARVIDSON G and T OLIVECRONA *Fatty acid composition of plasma and tissue lipids of normal and ethionine treated rats* Acta physiol scand 1962 55 303—312 — The fatty acid composition of plasma liver adipose tissue and carcass NFFA FFA and PLFA have been studied in normal and ethionine treated fasted female rats The fatty acid composition of adipose tissue and carcass lipids and also that of plasma FFA were uninfluenced by ethionine treatment Thus there was no sign of any qualitative difference in the mobilization of fatty acids from the depots in the ethionine treated rats The fatty acid composition of liver NFFA and PLFA and of plasma TGFA CEFA and PLFA on the other hand were significantly changed by ethionine treatment In the normal rats most of the ratios between the fatty acids were different in liver NFFA and adipose tissue NFFA In the ethionine treated rats the ratios not containing linoleic acid were not significantly different in liver NFFA and adipose tissue NFFA This agreement is taken as evidence for adipose tissue NFFA being the source of the increased liver NFFA As linoleic acid cannot be synthesized in the rat the linoleic acid which was present in the accumulated liver NFFA in an even higher percentage than in adipose tissue NFFA must also have derived from an extrahepatic tissue most probably adipose tissue

Ethionine the ethyl analogue of methionine induces fatty livers in fasted female rats (FARBER *et al* 19[illegible]) Liver cholesterol and phospholipids are within or near normal values whereas triglycerides are increased several fold

Abbreviations used

FFA — Free fatty acids
TGFA — Triglyceride fatty acids
NFFA — Neutral fat fatty acids
PLFA — Phospholipid fatty acids
CEFA — Cholesterol-ester fatty acids

(JENSEN *et al* 1951 HARRIS and ROBINSON 1961, OLIVECRONA 1962 a) Plasma esterified fatty acids are lowered (FEINBERG *et al* 1954 FURMAN *et al* 1957 HARRIS and ROBINSON 1961 OLIVECRONA 1962 a) whereas plasma FFA are elevated (HARRIS and ROBINSON 1961, OLIVECRONA 1962 a) Livers of ethionine treated rats take up and esterify plasma FFA at a rate similar to normal animals, but the fractional turnover rate of liver NFFA is lower than normal (OLIVECRONA 1962 b)

In order to study more closely the alterations in lipid metabolism induced by ethionine we have studied the relative amounts of the major fatty acids of plasma liver adipose tissue and carcass lipids from normal and ethionine treated fasted female rats

Experimental

The experimental animals were female Sprague Dawley rats (obtained from AB Anticimex, Stockholm Sweden) reared on a standard pellet diet[1] The rats weighed 160—190 g at the time of the experiment They were fasted for 16 hours then given a single intraperitoneal injection of 200 mg of ethionine in 8 ml half strength saline and fasted for an additional 24 hours They were then sacrificed by exsanguination. Plasma (arterial) liver and periovarian adipose tissue samples were obtained and analyzed as previously described (OLIVECRONA 1962 c) The remains of the rat were homogenized in a Waring blendor in 1 000 ml ethanol The homogenate was diluted to 4 000 ml by adding diethyl ether and allowed to stand at room temperature overnight. An aliquot was taken to dryness dissolved in chloroform methanol 2 1 (v v) and washed free of non lipid material by equilibrating against 0 4 volumes of 2 KH_2PO_4 in water The lipid extracts from plasma liver adipose tissue and carcass were then separated into their major classes using silicic acid chromatography as previously described (OLIVECRONA 1962 c) Free fatty acids were separated from neutral fat on ion exchange columns as described by CARLSSON and WADSTROM (1958)

The neutral fat fractions were saponified by refluxing in 3 % ethanolic KOH for 2 hours Hydroquinone was added to the saponifying medium to prevent loss of poly unsaturated acids (BOTTCHER *et al* 1959) After extraction of the unsaponifiable matter and acidification the fatty acids were extracted into petroleum ether taken to dryness and converted into the methyl esters using boron trifluoride in methanol as described by METCALFE and SCHMITZ (1961) The methyl esters were extracted into petroleum ether and dried over anhydrous Na_2SO_4 The free fatty acids obtained from the ion exchange columns were methylated directly with the boron trifluoride reagent as described above

The phospholipid fractions were transmethylated by refluxing for 2 hours in dry methanol with sulfuric acid as an interesterification catalyst The methyl esters were extracted into petroleum ether and dried over anhydrous Na_2SO_4

All samples were stored in solution at — 20 C until analyzed

The methyl esters were analyzed by gas chromatography using a Pye Argon Chromatograph which was equipped with a β ray ionization detector Column packing consisted of 10 % polyethylene glycol succinate on 80—100 mesh acid washed Celite 545 The polyester was synthesized under the conditions described by JAMES (1959)

[1] These pellets contained 3 4 fat The fatty acid composition was 16 0 19 2 % 16 1 1 2 % 18 0 2 6 18 1 3 8 18 2 48 3 18 3 and/or 20 0 5 1 %

Table I The percentage fatty acid composition (by weight) of liver fat fractions from normal and ethionine treated fasted female rats

	Normal						Ethionine treated					
	1	2	3	4	5	Mean	6	7	8	9	10	Mean
FFA												
16 0	52.3	48 8	45 2	48 7	51 8	49 4	38 4	38 9	42 9	44 4	42.5	41 4
16 1	2.5	1.5	1.5	1.5	1 1	1 6	1 9	2.1	1 3	1 4	1 6	1 7
18 0	10 1	9 0	9 6	8 1	8.3	9 0	9 1	11 8	13 4	11 8	11 5	11.5
18 1	24 0	27 8	30 3	28 6	26 8	27 5	32 1	32 4	29 0	28 6	29 4	30 3
18 2	10 9	13 1	13 4	12 9	11 8	12 4	18 5	14 7	13 3	14 0	15 0	15 1
20 4	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
NFFA												
16 0	24 5	27 8	34 4	30 6	30 0	30 1	20 8	23 1	21 1	25 0	[illegible]	22.5
16 1	2.5	4.3	2 0	3 4	3 1	3 1	6 3	3 9	2 7	2 2	3 1	3 6
18 0	9 2	7 4	7 3	9 0	4 8	7.5	3 2	2 6	3 1	4 1	3 4	3 3
18 1	25 2	25 7	23 1	23 6	24 3	24 4	33 3	33 5	30 6	29 0	33 7	32 0
18 2	30 6	30 2	28.0	27 0	29 1	29 2	32 6	34 7	38 7	33 6	34 8	34 9
20 4	7 8	4.3	5 3	6 4	5 1	5 8	3 6	2 4	3 8	6 1	2 5	3 7
PLFA												
16 0	21 7	21 1	24 7	25 7	21 8	23 0	16 6	17.2	—	17 9	16 9	17 2
16 1	tr	0 8	tr	tr	tr	tr	tr	tr	—	tr	tr	tr
18 0	33 7	31 5	31 0	30 9	31 6	31 7	33.5	28 9	—	34.9	34 4	32 9
18 1	6.2	7 6	6 6	6 6	8 9	7 2	11 0	13 0	—	8.7	9 8	10 6
18 2	14 0	13 5	15.5	13 9	14 7	14 3	19 7	23 3	—	19 0	20 7	20 7
20 4	24.4	25 3	22.3	22 8	22 8	23 5	19 2	17 6	—	19 5	18.2	18 6

nd = not determined tr = trace.

using succinic acid instead of adipic acid The peaks on the chromatograms were identified by comparing their retention time with those of known standards Peak areas were calculated by triangulation The designation of the fatty acids gives number of carbon atoms and double bonds (FARQUHAR et al 1959)

Results

In order to minimize the handling of the lipids prior to the gas chromatography analyses no determinations of the concentrations of lipids in the fractions containing small amounts of material (i e plasma lipids tissue FFA) were carried out However determinations of the liver lipids of these rats gave values similar to those previously found (OLIVECRONA 1962 a) on identically treated rats These values may therefore be taken as an approximation of the tissue lipid contents of the rats in the present study

Since the purpose of this investigation was to get a rough estimation of the change that ethionine might induce in the relative distribution of the major

Table II The percentage fatty acid composition (by weight) of adipose tissue fat fractions from normal and ethionine treated fasted female rats

	Normal						Ethionine treated					
	1	2	3	4	5	Mean	6	7	8	9	10	Mean
FFA												
16 0	32 6	32 0	35 0	35 0	35 6	34 0	34 8	35 2	35 0	33 0	35 2	34 6
16 1	1 9	2 7	3 4	2 3	2 3	2 5	1 8	2 3	1 6	2 2	1 7	1 9
18 0	7 0	6 9	6 6	8 6	8 5	7 5	8 8	9 5	9 1	7 4	8 8	8 7
18 1	38 8	38 5	38 5	37 4	38 6	38 4	37 9	37 0	37 0	39 4	38 7	38 0
18 2	20 0	20 0	16 8	16 7	15 3	17 8	17 0	15 8	17 3	18 1	15 6	16 8
20 4	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
NFFA												
16 0	28 1	26 2	28 7	29 2	28 4	28 1	30 0	30 3	24 0	26 4	25 6	27 3
16 1	4 1	4 3	3 7	3 8	4 4	4 1	3 9	4 2	3 4	3 6	4 4	3 9
18 0	4 4	3 8	4 7	4 8	4 3	4 4	4 4	4 4	5 3	4 1	4 8	4 6
18 1	36 1	35 4	34 6	37 7	38 2	36 4	37 1	34 9	36 0	37 2	36 4	36 3
18 2	27 3	30 2	28 4	24 6	24 9	27 1	24 9	26 2	31 4	28 9	28.8	28 0
20 4	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

nd = not determined.

fatty acids only the peak areas of palmitic palmitoleic stearic oleic linoleic and for some fractions arachidonic acid were calculated Thus the fatty acid spectra given here include only the major fatty acid components

The statistical analysis of the data have been performed using the Student t test Differences having a probability of less than 2 % of being due to chance have been designated as significant

Liver lipids (Table I)

NFFA The neutral fat fraction contained the cholesterol ester and the glyceride fatty acids Both in the normal and the ethionine treated rats however glyceride is the major fraction (OLIVECRONA 1962 a) so that the fatty acid composition of the neutral fat fraction may probably be taken as that of the glyceride DITTMER and HANAHAN (1959) reported that the liver cholesterol esters contain more arachidonic and stearic acid but less linoleic acid than the liver glyceride and that the percentages of palmitic and oleic acids were similar for the cholesterol ester and glyceride fatty acids

In the normal livers the most abundant fatty acids in the neutral fat fraction were palmitic, linoleic and oleic acids In the ethionine fatty livers there was a significant increase in the percentages of linoleic and oleic acids and a significant decrease in the percentages of palmitic and stearic acids However the amount of NFFA in the livers of the ethionine treated rats was about

Table III The percentage fatty acid composition (by weight) of carcass fat fractions from normal and ethionine treated fasted female rats

	Normal						Ethionine-treated					
	1	2	3	4	5	Mean	6	7	8	9	10	Mean
FFA												
16 0	37.6	34.9	35.5	35.6	35.1	35.7	36.8	35.8	36.4	35.8	32.2	35.4
16 1	4.2	4.0	4.3	4.6	4.0	4.2	4.1	4.4	4.2	4.3	3.9	4.2
18 0	8.8	10.5	9.7	11.5	10.7	10.2	9.8	9.7	11.2	9.0	10.4	10.0
18 1	6.7	35.7	35.6	32.3	33.8	34.8	37.4	34.8	33.2	32.7	34.0	34.4
18 2	12.7	14.9	14.7	16.1	16.4	15.0	11.9	15.3	15.0	18.1	19.5	16.0
20 4	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
NFFA												
16 0	0.0	23.0	25.2	24.4	26.6	25.8	27.0	23.3	31.1	22.8	25.5	25.9
16 1	4.4	3.9	3.8	4.9	3.6	4.1	4.4	4.2	3.1	3.8	3.8	3.9
18 0	5.5	4.6	4.6	4.9	4.9	4.9	3.9	4.5	4.3	4.1	4.3	4.2
18 1	42.0	40.3	37.4	39.1	38.4	39.4	36.9	40.0	31.7	40.0	37.2	37.2
18 2	18.1	28.0	28.9	26.7	26.4	25.6	28.0	27.8	29.8	29.3	29.4	28.9
20 4	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
PLFA												
16 0	32.3	30.3	29.8	36.8	34.1	32.7	31.3	29.2	33.8	34.2	29.2	31.5
16 1	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
18 0	20.6	23.5	20.1	19.2	19.4	20.6	17.3	21.5	19.2	18.2	22.0	19.6
18 1	17.6	17.4	17.6	19.4	19.8	18.4	21.7	17.5	18.8	20.0	19.7	19.5
18 2	18.2	16.7	17.4	14.9	15.2	16.5	19.5	17.6	16.6	18.3	17.2	17.8
20 4	11.6	12.1	14.9	9.5	11.4	11.9	10.1	13.9	11.5	9.3	11.8	11.3

nd = not determined.

6-fold increased (OLIVECRONA 1962 a) Thus there was an increase in all the fatty acids this increase being most marked for oleic and linoleic acids and least for palmitic and stearic acids

FFA In the normal rats palmitic acid was the most abundant liver FFA followed by oleic and linoleic acids In the ethionine treated rats the percentage of palmitic acid was significantly lowered whereas there were slight increases in the percentages of oleic linoleic and stearic acids

PLFA The most abundant liver PLFA in the normal rats was stearic acid followed by arachidonic and palmitic acids In the ethionine treated rats there were significant decreases in the percentages of palmitic and arachidonic acids and significant increases in oleic and linoleic acid percentages Since there was little or no change in the content of PLFA in the livers of the ethionine treated rats (OLIVECRONA 1962 a b) these changes reflect changes in total liver PLFA composition

Table IV The percentag fatty acid composition (by weight) of plasma fat fractions from normal and ethionine treated fasted female rats

	Normal						Ethionine treated					
	1	2	3	4	5	Mean	6	7	8	9	10	Mean
FFA												
16 0	33.0	31.2	—	35.6	35.4	33.8	33.2	31.6	0.6	37.1	38.1	34.1
16 1	3.0	2.7	—	3.5	2.1	2.8	2.9	4.1	3.0	3.2	3.5	3.3
18 0	10.6	8.9	—	9.1	9.9	9.6	8.4	8.1	10.1	8.1	8.5	8.6
18 1	34.0	38.5	—	33.2	36.0	35.4	36.2	36.0	36.8	34.4	32.4	35.2
18 2	19.4	18.7	—	18.5	16.7	18.3	19.4	20.2	19.6	17.2	17.6	18.8
20 4	nd	nd	—	nd	nd	nd	nd	nd	nd	nd	nd	nd
TGFA												
16 0	33.4	32.4	31.6	31.6	29.5	31.7	39.3	48.2	34.2	39.2	—	40.2
16 1	4.0	3.2	5.6	3.9	3.5	4.0	7.5	9.7	13.2	11.2	—	10.4
18 0	5.9	4.9	5.0	5.0	3.7	5.3	10.6	9.3	9.9	10.3	—	10.0
18 1	27.8	38.3	23.2	24.6	30.5	28.9	33.2	19.6	25.4	27.2	—	26.4
18 2	19.5	14.7	23.2	22.9	24.7	20.9	9.4	13.2	17.4	12.2	—	13.1
20 4	9.4	6.6	11.5	12.4	6.2	9.2	tr	tr	tr	tr	—	—
CEFA												
16 0	17.9	—	19.4	19.5	13.8	17.7	38.3	34.8	29.6	39.5	44.4	37.3
16 1	2.5	—	4.9	4.1	3.2	3.7	9.0	9.2	8.3	5.0	4.9	7.3
18 0	3.5	—	8.8	4.1	2.6	4.8	11.2	8.6	9.0	8.2	5.9	8.6
18 1	18.0	—	13.7	12.6	8.7	13.3	18.9	18.2	17.6	29.8	36.5	24.2
18 2	11.4	—	14.7	14.3	14.0	13.6	10.9	15.5	18.8	17.4	8.4	14.2
20 4	46.6	—	38.5	45.4	57.7	47.1	11.6	13.8	16.7	tr	tr	8.4
PLFA												
16 0	40.2	35.0	41.0	40.5	35.2	38.4	32.9	37.5	36.2	38.1	35.6	36.1
16 1	1.3	tr	tr	tr	tr	—	tr	tr	tr	tr	tr	—
18 0	30.1	36.2	31.2	33.8	25.6	31.4	21.6	21.3	22.6	16.3	18.4	20.0
18 1	14.4	11.4	10.7	12.5	22.7	14.3	17.6	14.1	12.7	16.4	12.3	14.6
18 2	14.1	17.4	17.2	13.3	16.5	15.7	28.1	27.1	28.4	29.4	33.8	29.4
20 4	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

nd = not determined tr = trace.

Adipose tissue lipids (Table II)

NFFA The most abundant NFFA in the adipose tissue were oleic palmitic and linoleic acids There was no significant change in the ethionine treated rats.

FFA There was more palmitic and less linoleic acid in the adipose tissue FFA than in the adipose tissue NFFA There was no significant change in the ethionine treated rats

Carcass lipids (Table III)

NFFA The composition of the carcass NFFA did not differ significantly from that of the adipose tissue NFFA There was no significant change in the ethionine treated rats

PLFA The most abundant carcass PLFA was palmitic acid followed by stearic oleic linoleic and arachidonic acids There was no significant change in the ethionine treated rats

Plasma lipids (Table IV)

CEFA The most abundant fatty acid in the plasma CEFA in the normal rats was arachidonic acid accounting for approximately 50 % of the total CEFA. The next most abundant fatty acid was palmitic followed by oleic and linoleic acids In the ethionine treated rats there was a drastic decrease in arachidonic acid All other fatty acid percentages were increased palmitic palmitoleic stearic and oleic acids all by factors of approximately 2 linoleic acid however only very slightly As the concentration of plasma CEFA in the ethionine treated rats was decreased (OLIVECRONA 1962 a b) there was probably a decrease in all plasma CEFA

TGFA The most abundant fatty acid in the plasma TGFA in the normal rats were palmitic oleic and linoleic acids In the ethionine treated rats there were significant increases in palmitic palmitoleic and stearic acid percentages and significant decreases in linoleic and arachidonic acid percentages

FFA The most abundant plasma FFA was oleic acid closely followed by palmitic acid. Also present were linoleic stearic and palmitoleic acids There was no significant change in the ethionine treated rats

PLFA The most abundant fatty acid in the plasma PLFA in the normal rats were palmitic and stearic acids In the ethionine treated rats there was a significantly decreased percentage of stearic acid and a significantly increased percentage of linoleic acid

Discussion

Liver fat accumulation can result from increased synthesis and/or uptake of fatty acids by the liver or from decreased disposal of fatty acids by the liver or from a combination of these two mechanisms In previous papers we have shown that it is quantitatively possible that all the accumulated fatty acids in ethionine treated rats are derived from adipose tissue via the plasma FFA (OLIVECRONA 1962 a b) despite the rate of mobilization of fatty acids being close to the normal one ARTOM (1959) has shown that livers from ethionine treated rats show decreased ability to oxidize fatty acids and we have shown that the rate of transport of fatty acids from the livers is also decreased Therefore available evidence suggests that the mobilization of

fatty acids from the adipose tissue is normal in ethionine treated rats and that the major cause of the liver fat accumulation is that the liver triglycerides are relatively unavailable both for oxidation and for transport to the plasma. The data are considered with respect to this hypothesis.

There were no significant differences between the fatty acid composition of adipose tissue lipids in the ethionine treated and the normal rats. The carcass lipids were also similar in the two groups of animals. The plasma FFA were alike in composition. Thus there was no evidence for any qualitative difference in the mobilization of fatty acids from adipose tissues in the ethionine treated rats.

In the liver and plasma esterified fatty acid fractions the ethionine treated rats showed marked differences from the normals. In the normal rats the fatty acid composition of liver NFFA and plasma TGFA showed many similarities. In the ethionine treated rats these fractions no longer resembled each other and both differed significantly from the respective fraction in the normal rats. Interestingly, the changes went in opposite directions. For example the percentage of palmitic acid was increased in plasma TGFA but decreased in liver NFFA, the percentage of linoleic acid was decreased in plasma TGFA but increased in liver NFFA. Arachidonic acid, however, was decreased in both liver NFFA and plasma TGFA and was also decreased to about 1/5 of its normal value in the plasma CEFA. The composition of both liver and plasma PLFA was markedly changed in the ethionine treated rats, in both cases towards decreased saturation.

We thus find that ethionine does not seem to interfere with the mobilization of fatty acids with regard to composition but significantly changes the fatty acid spectrum of liver and plasma lipids. This is in good accordance with the results of our previous studies on ethionine fatty livers (OLIVECRONA 1962 a, b). We have previously shown that it is quantitatively possible that all the fatty acids accumulating in the livers of ethionine treated rats may derive from adipose tissue (OLIVECRONA 1962 a, b). It is therefore of interest to find whether or not the present results support the hypothesis that the accumulating fatty acids do actually derive from adipose tissue. BRODIE *et al.* (1961) have attempted to compare the fatty acid composition of the additional liver fatty acids in the neutral fraction with adipose tissue NFFA in rats with ethanol induced fatty liver and found that these compositions coincided. Although the validity of any such calculation rests on numerous assumptions we have nonetheless made similar calculations on the present rats. In a preliminary calculation performed essentially as described by BRODIE *et al.* (1961) it was found that the composition of the additional fatty acids in the liver NFFA of the ethionine treated rats did not differ significantly from the composition of the total liver NFFA. The composition of the liver NFFA does not, however, coincide with that of the adipose tissue NFFA. There was less palmitic, stearic and oleic acids but more linoleic acid in the liver than in the adipose tissue

Table V Ratios between the fatty acids in liver NFFA and in adipose tissue NFFA

Ratio	Normal		P	Ethionine treated		P
	Adipose tissue NFFA	Liver NFFA		Adipose tissue NFFA	Liver NFFA	
16.1/16.0	0.14±0.01	0.104±0.04	0.05 < P < 0.10	0.14±0.02	0.14±0.13	No difference
18.0/16.0	0.16±0.01	0.26 ±0.09	0.02 < P < 0.05	0.17±0.03	0.14±0.02	0.05 < P < 0.10
18.1/16.0	1.29±0.06	0.82 ±0.15	P < 0.001	1.34±0.14	1.43±0.16	0.30 < P < 0.40
18.0/16.1	1.10±0.17	2.55 ±1.07	0.001 < P < 0.01	1.19±0.71	1.05±0.53	0.60 < P < 0.70
18.1/16.1	9.02 ±0.62	8.49 ±2.22	0.60 < P < 0.70	9.40±1.09	9.85±3.03	0.[illegible]0 < P < 0.80
18.1/18.0	8.31±0.79	3.41 ±0.97	P < 0.001	7.96±0.86	10.03±2.07	0.05 < P < 0.10
16.0/18.2	1.05±0.13	1.04 ±0.17	0.90 < P	0.98±0.19	0.65±0.07	0.001 < P < 0.01
16.1/18.2	0.15±0.02	0.105±0.03	0.02 < P < 0.05	0.14±0.02	0.106±0.05	0.20 < P < 0.30
18.0/18.2	0.17±0.03	0.26 ±0.06	0.01 < P < 0.02	0.16±0.02	0.095±0.02	P < 0.001
18.1/18.2	1.35±0.17	0.83 ±0.03	P < 0.001	1.30±0.13	0.92 ±0.09	P < 0.001

P represents probability for the difference between the ratios for liver and adipose tissue NFFA to be due to chance. Calculated using the Student t test.

NFFA However the ratios between the fatty acids for the liver NFFA and for the adipose tissue NFFA show some interesting relations (Table V) In the normal rats most of these ratios are different in the liver NFFA and the adipose tissue NFFA In the ethionine treated rats however the ratios not containing linoleic acid are not significantly different in the liver NFFA and the adipose tissue NFFA This agreement may be taken as evidence for adipose tissue NFFA being the source of the increased liver NFFA This reasoning cannot be applied to the linoleic acid which was present in the accumulated liver NFFA at even higher percentage than in the adipose tissue NFFA. Since linoleic acid cannot be synthesized in the rat (MEAD and HOWTON 1960) the additional linoleic acid in the liver NFFA of the ethionine treated rats must have derived from an extrahepatic tissue most pobably adipose tissue

If the fatty acids are transported to the liver from the adipose tissue this most probably takes place in the form of plasma FFA However these and also the adipose tissue FFA differed in fatty acid composition from the adipose tissue NFFA from which they were probably derived This might be due to the plasma FFA having some source other than adipose tissue NFFA. The most probable such source is the plasma TGFA (LAURELL 1959) Strong evidence against this possibility is the similarity of plasma FFA in normal and ethionine treated rats despite the latter having lower levels of TGFA which differed in fatty acid composition It is thus probable that during starvation plasma TGFA is not an important source of plasma FFA

We thus have the perplexing situation that the additional fatty acids taken up by the liver of the ethionine treated rats show many resemblances in their

fatty acid composition to the adipose tissue NFFA which most probably is their origin but show less resemblance with the plasma FFA, which most probably is the vehicle for their transport to the liver Interpretation of these differences must await more data on the turnover rate of individual fatty acids

The authors wish to express their appreciation to Mr WILFRIED POHL, Miss IRENE LINDELL and Miss AGNETA CELANDER for their skillful technical assistance and to Professor BENGT BORGSTROM and Dr ALAN HOFMANN for their help in preparing this manuscript.

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Acta physiol. scand 1962 55 313—318

From the Research Laboratories of the State Alcohol Monopoly (Alko) Helsinki Finland

Blood Sugar and Alcohol Intoxication in the Rat

By

LASSE SAMMALISTO

Received 23 December 1961

Abstract

SAMMALISTO L. *Blood sugar and alcohol intoxication in the rat* Acta physiol scand. 1962 55 313—318 — The effect of insulin and glucose on pharmacological ethanol intoxication was studied in rats by means of a tilting plane technique It was found that raised blood sugar tempered intoxication whilst lowered blood sugar enhanced it. Thus blood sugar is a factor affecting alcohol intoxication. In addition insulin seems to have a specific (enhancing) effect on alcohol intoxication not related to its effect on blood sugar

There is no complete agreement among investigators as to the influence of insulin and sugars — glucose has mainly been used — on the intoxicating effect of ethyl alcohol Most studies have dealt only with the effect on alcohol metabolism not with that on pharmacological intoxication In his review JACOBSEN (1951) concludes that both insulin and glucose are able to increase the rate of alcohol metabolism but that if the alcohol is already being oxidized at maximal rate the administration of these substances will have no effect on the blood alcohol curve

There are only a few investigations dealing with the effect of glucose on pharmacological intoxication In one of these (HAGGARD and GREENBERG 1938) it was found that glucose combined with alcohol caused a milder intoxication than did alcohol alone

Methods

In the tilting plane test devised in our laboratories (ARVOLA *et al* 1958 FRIEDMANN and INGALLS 1960 WALLGREN *et al* 1960) the animal is placed on a board with a rough surface On tilting the board the animal slides down at a certain angle. A sober rat can maintain its position at a steeper angle than an intoxicated one The performance during intoxication is expressed as a percentage of the sober value.

Table I Treatment in the different experiments A = alcohol G = glucose I = insulin. The indices refer to dosage alcohol and glucose in g/kg body weight insulin in int units Sex always male

Experiment no	No of rats	Source	No of replications in each treatment	Treatments	Test minutes after administration of alcohol
1	5	From 2 litters	1	$A_{4.5}$, $A_{4.5}I_{0.8}$, $A_{4.5}G_{4.5}$	90
2	5	From 2 litters	3	$A_{2.7}$, $A_{2.7}I_{0.4}$, $A_{2.7}I_{0.8}$	90
3	8	From 2 litters	3	$A_{5.4}$, $A_{5.4}I_{0.4}$, $A_{5.4}I_{0.8}$	
				$A_{5.4}$, $A_{5.4}I_{0.4}$, $A_{5.4}G$	90
4	24	From 5 litters	4	$A_{5.4}I_{0.4}G_{2.7}$ See Table IV	60 120 180 240 300 360 420 480 540
5					
a) Blood alcohol	44	From 9 litters	1	See Table V	60
b) Blood sugar	30	From 6 litters	1	See Table V	60
Total	116	From 30 litters			

Table II The influence of insulin on alcohol intoxication as measured by performance in the tilted plane test

Means for different treatments, expressed as percentages of the "sober" value

Alcohol g/kg	Insulin units	Replications 1	2	3	Total
2.7	0.0	86.6	90.2	86.4	87.7
	0.4	88.8	86.0	88.0	87.6
	0.8	83.0	86.8	84.8	84.9
5.4	0.0	84.4	68.0	78.4	69
	0.4	80.4	9.0	76.0	8.5
	0.8	78.8	70.0	65.0	71.3
Total	—	83.7	80.0	79.8	—

The investigation consisted of 5 series of experiments. In all cases the alcohol was given as a 30 per cent (v/v) solution in tap water by stomach tube. Glucose was given in a similar way. The insulin was injected subcutaneously in the neck. The animals were fasted overnight in the last experiment, but not in the others. The treatment in the different experiments are shown in Table I.

Table III The influence of insulin and glucose on alcohol intoxication as measured by performance in the tilted plane test Alcohol dosage 5 4 g/kg body weight

Means for different treatments, expressed as percentages of the sober value

Insulin units	Glucose g/kg	Replications			Total
		1	2	3	
0 0	0 0	75 3	76 8	84 3	78 8
	2 7	79 5	80 1	79 8	79 8
0 4	0 0	77 3	81 3	81 8	80 0
	2 7	89 8	85 0	93 1	86 0
Total	—	80 4	80 8	82.3	—

Results

The results of the first experiment suggested that insulin increases the pharmacological effect of alcohol whereas glucose seemed to do the opposite however the results were not statistically significant

In the second experiment the effect of insulin was studied in greater detail The results are presented in Table II from which it appears that insulin enhances alcohol intoxication but only if 0 8 unit is injected 0 4 unit seems to have no effect on alcohol intoxication (between insulin levels $p < 0\ 05$) The threshold for the effect of insulin is thus rather high

In the third experiment the aim was to ascertain the mode of interaction of alcohol insulin and glucose The results are given in Table III

The insulin dosage was low and had no effect on alcohol intoxication as was expected Nor did glucose alone have any effect but insulin combined with glucose tempered intoxication considerably (for insulin glucose inter action $p < 0\ 01$) This was interpreted to mean that high blood sugar makes the animal more resistant to alcohol but there remained the problem of why glucose alone did not have any effect on alcohol intoxication In order to resolve this contradiction a different timing of administration was applied in the fourth experiment For brevity s sake Table IV includes only the data for 1 and 3 hours after the administration of alcohol The whole curve is seen in Fig 1

Table IV shows that

— Insulin enhances alcohol intoxication considerably when given simultaneously with the alcohol when given one hour after alcohol administration it markedly retards recovery from the intoxicated state This fits in well with the results of the previous experiments

— Glucose has no effect on alcohol intoxication when given at the same time as the alcohol but if administered one hour earlier it considerably reduces

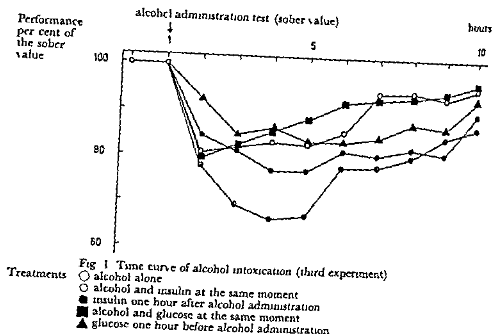


Fig 1 Time curve of alcohol intoxication (third experiment)

Treatments
○ alcohol alone
○ alcohol and insulin at the same moment
● insulin one hour after alcohol administration
■ alcohol and glucose at the same moment
▲ glucose one hour before alcohol administration

Table IV The effect of alcohol glucose, and insulin on performance on the tilting plane The dosages are 3 0 g alcohol (A) and glucose (G)/kg body weight and 0 4 units insulin (I)

	Time hours			
	0	1	2	4
1 Administration Performance	no —	A 100.0	no 81.0	no 83.2
2 Administration Performance	no —	A 100.0	I 84.4	no 77.1
3 Administration Performance	no —	A, I 100.0	no 78.1	no 66.5
4 Administration Performance	no —	A G 100.0	no 79.9	no 86.3
5 Administration Performance	G —	A 100.0	no 92.[illegible]	no 86.8
			3 significantly lower 5 higher than others	2 and 3 significantly lower than others

the degree of intoxication The failure to obtain a glucose effect in the earlier experiments was thus obviously due to wrong timing of its administration

The fifth experiment was designed to show whether the administration of insulin and glucose influences the blood sugar and blood alcohol curve As the

Table V The effect of alcohol glucose and insulin on blood alcohol and sugar and performance on the tilting plane The dosages are 3.0 g alcohol and glucose/kg body weight and 0.4 units insulin The test was made and blood samples taken 1 hour after administration of alcohol

	Mean test performance as percentage of the sober value	Mean blood alcohol mg	Mean blood sugar mg
Controls (C)	100.0	0	89
Alcohol alone (A)	84.6	160	96
Alcohol + glucose (AG)	93.5	115	127
Alcohol + insulin (AI)	74.9	154	77
	AG/A $P < .03$	AG/A $P < .001$	AG/A $P < .001$
	AI/A $P < .02$	AG/AI $P < .005$	AI/A $P < .001$
			AI/C $P < .04$

stress caused by withdrawing blood from the tail tip was suspected to raise the blood sugar the samples were taken from rats tested and killed one hour after the administration of the alcohol The samples were taken within half a minute of killing Blood alcohol was determined according to the method of NEWMAN and NEWMAN (1953) blood sugar by the method of SOMOGYI (1945) The results are presented in Table V

Glucose raised and insulin lowered the blood sugar as expected. Alcohol, too seemed to raise the blood sugar as already noted by VARTIA *et al* (1958) and LANGE and KUHNE (1960) but our result is not statistically significant in this respect

After the administration of glucose the blood alcohol level was significantly lower than when glucose was not given Insulin had no effect on blood alcohol The results in the tilting plane test agreed with previous findings The effect of glucose is thus *related to a decrease in the blood alcohol level whereas* insulin enhances intoxication but does not raise blood alcohol This suggests that insulin has a specific effect on alcohol intoxication but in addition it obviously has an effect on intoxication *via* its influence on the blood sugar level as seen from the results of the third experiment (p 3)

The material used for all experiments has been genetically heterogeonous and the experimental conditions have varied somewhat Nevertheless the result has always been the same insulin enhances the pharmacological effect of alcohol whilst glucose tempers it These effects are correlated with the blood sugar level but in addition insulin seems to have a specific effect on intoxication.

In this case the result is clear but it seems to imply that in all kinds of studies on alcohol intoxication the material should be both genetically and environmentally far more varied and the body of data, accordingly very large

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Acta physiol scand 1962 55 319—324

From the Department of Pharmacology University of Uppsala Sweden

Tubular Excretion of Serotonin (5-Hydroxytryptamine) in the Chicken

By

ERLAND SANNER and BERNARD WORTMAN[1]

Received 30 December 1961

Abstract

SANNER, E. and B WORTMAN *Tubular excretion of serotonin (5-hydroxytryptamine) in the chicken* — Acta physiol scand 1962 55 319—324 — Serotonin at a dose of 200 μg given to normal chickens by unilateral leg vein infusion showed an ipsilateral excess of excretion The excess excretion was below 10 per cent of the dose however Birds pretreated with a mono amine oxidase inhibitor showed an ipsilateral excess of such a magnitude (26 per cent of the injected dose) that it proves a tubular excretion of serotonin Reserpine failed to block this process but a small blocking effect of reserpine cannot be excluded Urine flow was not significantly altered by the dose of serotonin used in this study

Nothing is known about the renal excretion mechanism of serotonin (5-hydroxytryptamine) It has been shown by LINDAHL and SPERBER (1956 1958) that histamine is excreted by active tubular secretion in the chicken kidney It was therefore considered of interest to study the renal tubular handling of serotonin in the chicken

Since reserpine causes a release of serotonin by certain cells for instance blood platelets (CARLSSON SHORE and BRODIE 1957) it seemed of interest to test whether or not reserpine affects serotonin transport by tubular cells The influence of reserpine on renal excretion of serotonin was therefore studied

Excretion of serotonin might be affected by a simultaneous destruction by mono amine oxidase The influence of a mono amine oxidase inhibitor (Catron®) on the renal excretion of serotonin was therefore also studied

[1] Special Fellow of the National Institutes of Health United States Public Health Service Present address Washington University School of Medicine St. Louis 10 Missouri

Materials and Methods

The general procedure was that of CAMPBELL (1960) CAMPBELL and SJOBERG (1960) Rhode Island Red chickens about two years old weighing 2 4—3 4 kg were used They were kept on a commercial mesh and fresh water ad libitum Generally the birds were kept fasting overnight before the experiment The birds were unanesthetized and kept in the sitting position during the experiment Small plastic funnels were sutured over each ureteral orifice under topical anesthesia The funnels were irrigated with a constant flow of distilled water at a rate of 0 5 ml/min on each side to prevent clogging of the funnels by uric acid The urine was collected from each kidney separately All the birds were tested by injecting 0 2 mg/kg phenol red into one leg vein The very few birds that did not excrete phenol red with a clear excess on the injected side were discarded Serotonin creatinine sulphate (Sandoz) was provided in ampoules containing 10 mg/ml All serotonin solutions were diluted with 0 9 per cent aqueous saline to contain 100—200 μg/ml The serotonin was infused by a motor driven syringe into one leg vein through a polyethylene catheter at a dose of 200 μg/bird in all experiments (59—83 μg/kg body weight) The infusion was made over 10 min Reserpine (Serpedin© Pharmacia) was provided in ampoules containing 2 5 mg/ml and injected as such into the pectoral muscle or into the wing vein at a dose of 1 mg/kg The substance JB 516 (1 phenyl 2 hydrazinopropane Catron© Lakeside) was given at a dose of 10 mg/kg (10 mg/ml in 0 9 per cent aqueous saline) into the wing vein generally 1 hour sometimes 2—5 hours before the experiment The urine samples were kept in the refrigerator at + 4 C overnight and analyses for serotonin were made the following day by a fluorometric method described by BERTLER and ROSENGREN (1959) and BERTLER (1961) The fluorescence activity was read in an Aminco-Bowman spectrophotofluorometer The activating wave length was set to 300 mμ and the fluorescent wave length was 540 mμ The recovery of serotonin added to the dilute chicken urine was 109 per cent (1 expt) The difference between the amounts excreted by the ipsilateral and the contralateral kidney divided by the dose given was taken as a measure of the tubular excretion of serotonin This was called the apparent tubular excretion fraction (ATEF) according to LINDAHL and SPERBER (1958)

Results

A Serotonin given to normal birds

Serotonin at a dose of 200 μg infused into one leg vein for 10 min produced an increased output of serotonin in the urine on the ipsilateral side Almost no serotonin came out in the urine on the contralateral side The values on the ipsilateral side were very variable In 9 experiments the urine was collected with a somewhat different time schedule After 20 min of urine collection the average excess of excretion in per cent of dose given (ATEF 100) was 6 6 ± 1 7 (9 exp) In 6 of these experiments the urine collection was continued for 30 min from the start of infusion Here the corresponding figures were 7 6 ± 2 4 per cent In 4 out of these exp the collection was continued for 40 min Here the figures were 7 9 ± 3 2 per cent In one of these 4 exp the collection period was 50 min In this experiment the excess excretion was 3 6 per cent (Table I) Serotonin appeared in the urine after about 5 min of infusion In almost all experiments the peak excretion was in the collection

Table I

Number of exp. and av weight of birds	Serotonin excreted		Total recovery per cent of dose	Excess excretion per cent of dose (ATEF 100)	Collection period minutes
	Inj side μg	Non inj side μg			
	A. Serotonin				
9 (3 0 kg)	3 6 ± 1 2	0 3 ± 0 1	2 0 ± 0 6	1 7 ± 0 6	10
	11 0 ± 2 8	0 6 ± 0 2	5 8 ± 1 4	5 2 ± 1 4	15
	14 0 ± 3 4	0 8 ± 0 3	7 4 ± 1 7	6 6 ± 1 7	20
	15 5 ± 5 3 (6)	0 5 ± 0 1 (6)	8 0 ± 2 4 (6)	7 6 ± 2 4 (6)	30 (6)
	16 2 ± 6 3 (4)	0 5 ± 0 1 (4)	8.4 ± 3 2 (4)	7 9 ± 3 2 (4)	40 (4)
	7 7 (1)	0 5 (1)	4 1 (1)	3 6 (1)	50 (1)
	B Catron-serotonin				
15 (3 0 kg)	8 3 ± 2 5	0 7 ± 2 7	4 5 ± 1 3	3 8 ± 1 2	10
	33 2 ± 3 2	1 7 ± 0 6	17 5 ± 1 7	15 8 ± 1 6	20
	43 8 ± 4 5	2 5 ± 0 7	23 2 ± 2 3	20 7 ± 2 3	30
	51 7 ± 6 6 (10)	3 4 ± 1 3 (10)	27 6 ± 3 5 (10)	24 2 ± 3 3 (10)	40 (10)
	54 2 ± 7 6 (9)	4 3 ± 1 7 (9)	29 3 ± 4 1 (9)	24 9 ± 3 7 (9)	50 (9)
	57 9 ± 7 9 (9)	5 4 ± 2 2 (9)	31 9 ± 4 1 (9)	26 0 ± 3 7 (9)	70 (9)
	C Reserpine serotonin				
2 (2.8 kg)	2 7	0 5	1 6	1 1	10
	6 7	1 1	4 0	2 9	15
	15 8 (1)	4 3 (1)	10 1 (1)	5 8 (1)	50 (1)
	D Reserpine-catron serotonin				
8 (2 7 kg)	6 6 ± 2 7	0 7 ± 0 1	3 7 ± 1 5	3 0 ± 1 2	10
	25 5 ± 5 3	1 9 ± 1 1	13 7 ± 3 0	11 8 ± 2 4	20
	33 6 ± 5 8	2 9 ± 1.2	18 0 ± 3 2	[illegible] ± 2 7	30
	37 2 ± 6 1	3 8 ± 1 3	20 5 ± 3 4	[illegible]	40
	39 8 ± 6 2	4 7 ± 1 4	22 3 ± 3 4	[illegible]	[illegible]
	43 0 ± 5 7	6.5 ± 1 6	24 8 ± 3 3	18 3 ± 2 7	70

Figures in parenthesis indicate number of experiments when it was less than the number given in the first column.

period just after the end of infusion Forty minutes after the end of infusion almost no further serotonin was excreted Urine flow was not altered significantly Electrolytes were not analysed

In 2 exp (not shown) infusion was performed first into one leg and then after 3 hours into the other Total recovery and excess excretion on the ipsilateral side were only about half as large after the second infusion than after the first

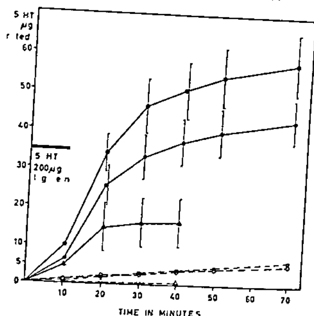


Fig 1 Urinary excretion of serotonin after unilateral leg vein infusion of 200 g serotonin (5-HT) to chickens Serotonin (4 birds) ▲—▲ infused side △—△ control side Catron serotonin (9 birds) — infused side — control side Reserpine-catron serotonin (8 birds) ■—■ infused side □ □ control side The points given represent the means and the vertical bars their standard errors.

B Serotonin given to birds pretreated with a mono amine oxidase inhibitor

In 15 exp urine was collected for 30 min from the start of the infusion of serotonin The mean excess excretion was 15 8 ± 1 6 per cent of the dose at 20 min and 20 7 ± 2 3 per cent at 30 min In 9 of these experiments collection was continued to a total of 70 min These gave a mean excess excretion of 26 0 ± 3 7 per cent In catron pretreated birds the excretion of serotonin in the urine continued for a somewhat longer time than in normal birds Total recovery was markedly increased and some more escaped out on the contra lateral side compared to normal birds (Table I) Urine flow was not significantly altered Electrolytes were not measured

C Serotonin infusion in previously reserpinized birds

In 2 exp reserpine was administered as follows First 1 mg/kg reserpine was given into the pectoral muscle 17 hours before the experiment An additional dose of 1 mg/kg reserpine was given into the wing vein 20 min before the infusion of serotonin Thereafter 200 µg of serotonin was infused into one leg vein for 10 min In these two experiments urine was collected for 15 min after the start of serotonin infusion The excess excretion in per cent of dose given (ATEF 100) was 2 0 and 3 7 In the second of these experiments urine

collection continued for a total of 50 min after the start of the infusion At that time the excess excretion was 5 8 per cent With the corresponding dose and timing of serotonin infusion in a normal bird we got the figures 5 2 $\pm$ 1 4 per cent at 15 min (9 exp) and 3 6 per cent at 50 min (1 exp) respectively

D Serotonin infusion to previously reserpinized birds pretreated with a mono amine oxidase inhibitor

The same dose 200 μg of serotonin was infused for 10 min to chickens that received 1 mg/kg reserpine 17 hours before the experiment One hour before the infusion started the bird was given 10 mg/kg catron into the wing vein Eight experiments were carried out The urine was collected for 70 min after the start of serotonin infusion At 20 30 and 70 min urine collection time the excess excretion in per cent of dose given (ATEF 100) was 11 8 $\pm$ 2 4 15 2 $\pm$ 2 7 and 18 3 $\pm$ 2 7 respectively (Table I) The excretion pattern resembled closely that of the other catron experiment reported but the values for total recovery and excess excretion were somewhat lower

Discussion

The figure and the table summarize the main results Unilateral infusion of 200 μg serotonin into a normal bird gave an easily measured excretion on the ipsilateral side with very small amounts of serotonin appearing in the urine on the contralateral side The clearcut excess of excretion on the infused side pointed to a tubular secretion but the recovery of the dose given was too small definitely to allow such a statement The excess excretion of the dose given was less than 10 per cent and could therefore also have been due to a simple diffusion process in the infused kidney (SPERBER 1948 b) After infusion of serotonin in one leg vein and thereafter repeating the same infusion on the other side total recovery and excess excretion were only about half of that after the preceeding infusion procedure If this phenomenon (observed in only two experiments) is not due to chance its explanation is not apparent

On the birds pretreated with catron the infused serotonin was excreted on the ipsilateral side to a much higher extent than in normal birds The excretion on the contralateral side was still very small but some more escaped out on this side The excess excretion in per cent of dose given (ATEF 100) was 26 0 $\pm$ 3 7 when the urine was collected for 70 min This shows that the tubular excretion of serotonin could not be studied in a normal bird because of the large breakdown of the injected substance in the kidney

The figures of excess excretion strongly point to the conclusion that serotonin is excreted by tubular excretion

Pretreatment with 1 mg/kg reserpine the previous day and an extra dose of reserpine just prior to the infusion of serotonin was tried in two experiments only The excretory pattern of serotonin in these two experiments pointed in-

the direction that reserpine might inhibit the tubular excretion but the number of experiments was too small and the great variability of the excretion figures in normal birds made it impossible to draw such conclusions

If a previously reserpinized bird was treated with a mono amine oxidase inhibitor prior to the experiment the excretion pattern was similar to that in birds treated with catron only, but the total recovery and excess excretion were slightly less This suggested a possible inhibition by reserpine of the excretion mechanism for serotonin, which however did not stand up to statistical tests It is possible that serotonin is excreted by two mechanisms by an unspecific transport system common to many bases and by a reserpine sensitive one Further work is, however, needed to settle this question

Added in proof

This excess excretion of serotonin on the ipsilateral side could also be shown to be true in birds treated with soldium bicarbonate to make the urine alkaline to pH 8 Thus the tubular excretion of serotonin is not due to pH dependent non ionic diffusion and can be considered to be an active one

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Serotonin (serotonin creatinine sulphate) was kindly supplied by Sandoz Inc Basel reserpine (serpedin©) by Pharmacia Inc Uppsala and the substance JB-516 (catron©) by Lakeside Laboratories Inc Milwaukee Wisconsin

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From the Department of Clinical Chemistry and the Department of Neurological Surgery, University of Lund, Sweden

The Solubility of Carbon Dioxide in Cerebral Cortical Tissue of Cats

With a note on the solubility of carbon dioxide in water, 0.16 M NaCl and cerebrospinal fluid.

By

BO K. SIESJÖ

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Abstract

SIESJÖ, B. K. *The solubility of carbon dioxide in cerebral cortical tissue of cats.* Acta physiol. scand. 1962. 55. 325—341. — The solubility of carbon dioxide in cerebral cortical tissue from cats was determined in homogenates acidified to $\mathrm{pH} < 4$ (*cf.* VAN SLYKE, SENDROY, HASTINGS and NEILL, 1928). The homogenates, which were prepared from brains frozen *in situ* with liquid nitrogen, were equilibrated with pure carbon dioxide at 37.5° C. The carbon dioxide content of the homogenates was determined by the microdiffusion method of CONWAY (1950) and the resulting KUENEN coefficients (ml CO_2/g and ml CO_2/g of H_2O) calculated. A linear relationship was found between solubility coefficient and tissue concentration in the homogenates. Extrapolation to undiluted tissue gave the coefficients 0.49 (ml CO_2/g tissue) and 0.61 (ml CO_2/g of H_2O in the tissue) respectively. The solubility of carbon dioxide in the tissue followed HENRY's law, which should indicate that no appreciable amount of carbon dioxide is adsorbed onto tissue constituents. Measurement of the solubility coefficient for carbon dioxide in cerebrospinal fluid at 37.5° C revealed that this coefficient came very close to that obtained in a 0.16 M NaCl solution. The solubility coefficient in water, originally determined to test the methods used, was in good agreement with two, but in less good agreement with the majority of the coefficients reported in the literature. An examination of the literature indicated a source of error in the determination of the latter values.

A better understanding of the manifold and important effects of carbon dioxide on the central nervous system requires knowledge of the relations within the bicarbonate/carbonic acid buffer system of the tissue Such knowledge is pertinent not only to studies of possible individual effects exerted by the components involved in the buffer system but also to studies of acid base shifts in the tissue

The HCO_3/H_2CO_3 buffer of liquids, such as blood, has been thoroughly analysed In such studies, the relation between the components of the buffer system has usually been expressed in the familiar HENDERSON HASSELBALCH equation

$$pH = pK_1 + \log \frac{(HCO_3)}{(H_2CO_3)} = pK_1 + \log \frac{(T_{CO}) - (P_{CO}\ S)}{(P_{CO}\ S)} \qquad (1)$$

where pK_1 is the first apparent dissociation constant of carbonic acid, P_{CO} the carbon dioxide tension S a solubility factor (HENRY s law constant), and T_{CO} the total carbon dioxide content of the solution ($CO_2 + H_2CO_3 + HCO_3$) In formulating the equation for use in a biological system it is assumed that the solubility of carbon dioxide follows HENRY s law, and further, that no complex bound carbon dioxide (like carbamino CO_2) exists Provided the constants are known the equation will make it possible to calculate one of the factors pH P_{CO} or HCO_3^- if the other two (or T_{CO}) are measured It has been shown using blood plasma that such a procedure is valid only when the experimental conditions are rigidly controlled and the constants accurately determined (SEVERINGHAUS STUPFEL and BRADLEY 1956)

A corresponding analysis of the HCO_3^-/H_2CO_3 buffer system of a tissue such as the cerebral cortex is far more difficult In the first place neither constants nor values measured can be referred to a single compartment in the tissue Secondly even if the tissue is tentatively divided into compartments no direct measurement of tissue pH can be carried out These difficulties are reflected in the sparse literature on the subject In fact the only variable which has been studied directly is the total carbon dioxide content of tissue samples obtained after decapitation of experimental animals (BRODIE and WOODBURY 1958 NICHOLS 1958 THOMPSON and BROWN 1960 KOCH and WOODBURY 1960) It is debatable however whether these measurements do represent actual *in vivo* conditions Thus the carbon dioxide content must be influenced by the kind and concentration of the buffer anions in the tissue These anions are composed partly of labile tissue metabolites the concentration of which change markedly in few seconds after decapitation (cf MCILWAIN 1959) Moreover the tissue metabolism gives rise to an accumulation of carbon dioxide in the period between decapitation and analysis It is thus evident that as long as the post mortem changes cannot be adequately controlled the total carbon dioxide values must be treated with caution when used for quantitative calculations

A still greater uncertainty is introduced when the values for total carbon dioxide content are used for calculations of the acid base metabolism of the tissue In previous works it was necessary to assume that the tissue carbon dioxide tension was the same as that in arterial (or venous) blood and further that the pK_1 and the carbon dioxide solubility coefficient of blood plasma could be used for brain intracellular water (BRODIE and WOODBURY 1958 NICHOLS 1958 regarding the solubility see below) Further assumptions were introduced by the use of the chloride space to separate extra and intra cellular compartments

Although our knowledge of the HCO_3/H_2CO_3 buffer system of the cerebral cortex is meager and the measurements performed are open to theoretical objections accurate measurements of the total carbon dioxide content still represent a useful approach Thus the development of a method for quantitative measurements of the average carbon dioxide tension of the cerebral cortex (SIESJO 1961) affords a means of studying an important variable of the buffer system continuously *in vivo* Knowledge of the solubility of carbon dioxide in the tissue would make it possible to express the results of these measurements also in terms of carbonic acid concentration If then the total carbon dioxide content of the tissue is accurately determined a distinction can be made between the carbon dioxide dissolved and that combined chemically

The present experiments were prompted by the need for further work along the lines indicated above The experiments to be reported below were undertaken to analyse the HCO_3^-/H_2CO_3 buffer system of homogenized cerebral cortical tissue by determination of (1) the solubility of carbon dioxide (2) the amount of carbon dioxide bound by buffers at different carbon dioxide tensions (3) the apparent dissociation constant of carbonic acid and (4) the relation between pH and carbon dioxide tension During the study an attempt was made to minimize post mortem tissue changes by freezing the brains of cats *in situ* The subsequent homogenization of cortical tissue and equilibration with different carbon dioxide tensions were made in an anaerobic atmosphere in the presence of an inhibitor of tissue glucolysis Carbon dioxide values were expressed as amount per gram of tissue and per gram of water in the tissue respectively Thus no attempt was made to separate extra and intra cellular compartments Values for undiluted tissue were found by extrapolation from the homogenates

This paper deals with the solubility of carbon dioxide in cortical tissue Except for an isolated determination in fat free homogenates of rat brain and muscle tissue (NICHOLS 1958) no measurement of the solubility coefficient for carbon dioxide in a composite tissue seems to have been reported previously The significance of the BUNSEN coefficient obtained by NICHOLS (0 47 ml CO_2/ml of H_2O in the tissue) is questionable (see Discussion)

In the present work the homogenates were acidified and the KLEMEN coefficient (ml CO_2/g tissue and ml CO_2/g of H_2O in the tissue respectively) determined for homogenates containing from five to forty five per cent cells It will be shown that the solubility coefficient was linearly related to the per cent by weight of tissue in the homogenates After extrapolation to un diluted tissue the coefficients 0 49 (ml CO_2/g tissue) and 0 61 (ml CO_2/g of H_2O in the tissue) were obtained. Experiments performed with lower carbon dioxide tensions revealed that the solubility followed HENRY's law a fact which should exclude adsorption of carbon dioxide in the tissue under the experimental conditions chosen

Additional experiments were done to determine the solubility coefficient for carbon dioxide in 0 16 M NaCl, in cerebrospinal fluid and in water The experiments on water which served as a test of the methods used, clearly indicated that the majority of coefficients given in the literature are too high.

The solubility coefficient for carbon dioxide in tissue derived from the present experiments made it possible to study the amount of carbon dioxide bound by buffers at different carbon dioxide tensions the pK_1 of carbonic acid as well as the relation between pH and carbon dioxide tension in the homogenates The results of these experiments will be presented in subsequent reports (SIESJÖ 1962 b—c)

Theory

HENRY's law states that the amount of gas dissolved in a liquid is directly proportional to the partial pressure of the gas in equilibrium with the liquid At moderate carbon dioxide tensions the equation can be written

$$P_{CO_2} \cdot K = (CO_2) \qquad (2)$$

In contrast to most other gases carbon dioxide does combine to a certain extent with the solvent molecules



$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \quad K_h = a_{H_2CO_3}/a_{CO_2} = 0.00317 \text{ at } 38° \qquad (3)$$

In water solutions the physically dissolved carbon dioxide will thus exist partly as anhydrous partly as hydrated gas The hydrated form does, however form only a negligible part of the total carbon dioxide in solution (*cf* EDSALL and WYMAN 1958) and it is conventional to designate both forms either in terms of carbon dioxide or for acid base calculations in terms of carbonic acid

The solubility is usually expressed as a coefficient the two most widely used coefficients being *the* BUNSEN *absorption coefficient* (α) and *the* OSTWALD *solubility coefficient* (L or l) The BUNSEN coefficient (α) is defined as the ratio of the volume reduced to standard conditions of gas dissolved at 1 atm. to the volume of the solvent. This coefficient has been used to express the solubility of carbon dioxide in blood plasma (VAN SLYKE SENDROY HASTINGS and NEILL 1928 BARTELS and WRBITZKY 1960 erythrocytes (VAN SLYKE *et al* 1928) and body fat (NICHOLS 1957) If the coefficient

and the carbon dioxide tension are known the carbonic acid concentration can be calculated thus

$$H_2CO_3\,(\text{mM/l}) = \frac{p_{CO_2}}{760} \cdot \frac{\alpha}{22.26} \cdot 1000 \qquad (4)$$

22.26 being the volume of 1 mg molecule of CO_2 at 0° and 760 mm Hg (GUYE and PINTZA 1908).

In the majority of previous studies, the carbon dioxide dissolved has been measured by physical means. In calculating the coefficients, the volume of gas was reduced to standard conditions using the ideal gas laws. Thus, coefficients obtained by chemical methods can be expected to differ from those measured by physical methods in the same degree as the gas deviates from the ideal gas laws (MARKHAM and KOBE 1941).

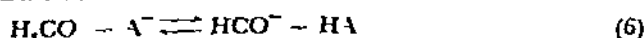
In the present work the carbon dioxide dissolved was chemically determined. The convention is, when dealing with a tissue like the cerebral cortex, to express concentrations per unit weight and not per unit volume. Accordingly the solubility was calculated as a BUNSEN coefficient, except that the amount of solvent was 1 g. The resulting coefficient is then in fact a KUENEN coefficient (l = ml CO_2/g). In accordance with previous work (VAN SLYKE *et al.* 1928) the coefficient obtained was given the subscript (l) when the carbon dioxide concentration was calculated per g of water in the solutions.

When carbon dioxide is dissolved in water solutions it does not only combine with the water molecules but also with the hydroxyl ions



$$CO_2 + OH^- \rightleftharpoons HCO_3^- \qquad (5)$$

The bicarbonate ions formed represent chemically combined carbon dioxide. As this reaction may be of importance when determining the solubility coefficient in water the procedure of acidifying the water and the salt solutions was followed (VAN SLYKE *et al.* 1928). The acidification becomes of paramount importance when working with biological fluids and tissues where bicarbonate ions are formed through the interaction of carbonic acid and different buffer anions (A^-)



$$H_2CO_3 + A^- \rightleftharpoons HCO_3^- + HA \qquad (6)$$

If the solutions are acidified to pH below 4 negligible amounts of bicarbonate can be formed and all the carbon dioxide in the solution can be looked upon as being dissolved. The addition of acids will however slightly influence the solubility of the gas. To account for this influence correction factors for the added ions have been determined (VAN SLYKE *et al.* 1928). These factors were used in the present work.

Methods and experimental techniques

The animal experiments were performed on eight cats after preliminary tests on rats and rabbits. The cats were anaesthetized with Nembutal (40 mg/kg body weight i.p.) and allowed to breathe spontaneously. The brain was exposed through a wide craniotomy. The dura was then resected carefully avoiding injury to blood vessels and venous sinuses. Liquid nitrogen was then immediately poured onto the brain until it was completely frozen. The frozen tissue was separated and placed in liquid nitrogen. The cortical tissue was then chiselled from the underlying white matter in a cold room (+ 4° C) and fragmented in a chilled stainless steel beaker. In separating the cortical tissue the frozen tissue fragments were cleft 1—2 mm from the surface of the gyri, and the pieces trimmed so as to remove all substance which was macroscopically white. The procedure of freezing *in situ* is that of KERR (1935).

During the whole process the frozen fragments of cortical tissue were not allowed to thaw until they were introduced into the icecold acid (0.1 N hydrochloric or previously boiled lactic acid) contained in a 5 ml POTTER glass homogenizer with a tightly fitting pestle. Prior to this the acid was equilibrated with nitrogen and the amount pipetted into the homogenizer was weighed by difference on an analytical balance. After the introduction of the cold pieces of tissue into the homogenizer the latter was closed with a rubber stopper leaving a small slit at the nozzle of the homogenizer to allow the expanding gas to escape. The tissue was then homogenized for 3—5 minutes, care being taken that all fragments of tissue were ground up. The surface of the glass homogenizer was carefully dried before the homogenizer was once again weighed to determine the amount of tissue. The latter was thus expressed as percentage of weight of the homogenate.

The water used was distilled once and acidified to a concentration of 0.1 N lactic acid or 0.1 N hydrochloric acid. For the determination of the solubility coefficient in water, the lactic acid solution or alternatively a 0.01 N HCl solution was used. Sodium chloride solutions of an ionic strength of 0.16 M were prepared from the analytical grade salt and 0.001 N HCl. Cerebrospinal fluid with a protein content of 20—40 mg per cent, obtained from humans by puncture, was acidified by adding 1 per cent concentrated lactic acid.

For equilibration with the gases used the homogenates and the solutions were transferred to ball tonometers (LAGÉ 1951) in a water bath, the temperature of which was kept at 37.5° C (± 0.1° C). To ensure that the gases attained the temperature of the water bath they were led through copper tubes before entering the bubble humidifiers. Double samples were taken after about 30 minutes and 40 minutes equilibration using ordinary straight glass pipettes with sufficient bore to permit a 0.1—0.3 ml sample to be pipetted in about 1—2 seconds. The pipettes, which were kept at the temperature of the water bath, were flushed several times with the equilibration gas before the samples were taken. The speed of the gas flow was increased during the sampling from the tonometer.

The gas used for equilibration was either carbon dioxide (KOLSYREFABRIKEN, Malmö) stated to be 99.7 per cent pure, or a carbon dioxide-nitrogen mixture. The carbon dioxide concentration of the latter mixtures was determined by SCHOLANDER analysis[1]. The steel cylinders containing the mixtures were kept horizontally. The mixtures usually contained 0.2—0.3 per cent oxygen, which was removed at 400° C in an electric oven by leading the gases through Pyrex glass tubes containing bright copper wires. The glass tubes were first packed with cupric oxide wires (BAKER) which were reduced by hydrogen gas during gentle heating[2].

The carbon dioxide tension was calculated according to the usual formula:

$$P_{CO_2} = \frac{\text{Per cent } CO_2}{100} \cdot (B - 48.4) \qquad (7)$$

where B is the corrected barometric pressure in mm Hg and 48.4 the vapour tension of water at 37.5° C. The barometric pressure was read from a mercury manometer immediately before each experiment.

The carbon dioxide content of the equilibrated samples was determined with the microdiffusion method of CONWAY (1950) using 2 A units (CALLENKAMP, London) and a 500 µl horizontal burette (DANSK LABORATORIEUDSTYR, Copenhagen).

[1] Performed at the Institute of Physiology, Lund.

[2] The method was suggested by Dr E. BLADH (Dept. of Analytical Chemistry, Lund), whose help is gratefully acknowledged.

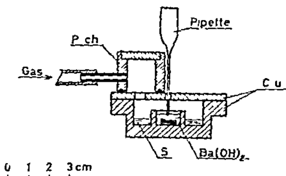


Fig 1 Conway unit (2A) arranged for perfus on with carbondioxide free nitrogen gas.

C u. = Conway unit with lid in place.

S = sample.

P ch. = perfusion chamber in steel with cover of Perspex and ring mounted at the lower end of the steel walls.

The influence of atmospheric carbon dioxide upon the measurements was avoided by a slight modification of that described by HOLM JENSEN (1961) Thus a second drill hole (5 mm in diameter) was made in the glass lid matching the outer chamber of the unit with the lid in place. This additional hole could be covered with a steel chamber for perfusing the unit with carbon dioxide — free nitrogen gas Contact was made between the perfusion chamber and the lid by means of an o-ring fitted into the lower end of the chamber (Fig 1)

The titration was performed as follows the unit and the periphery of the drill holes were coated with vaseline and the lids brought into place 0 3 ml 0 1 N HCl was pipetted into the outer chambers of the units Nitrogen gas was then passed through a carbon dioxide absorber and led in parallel streams through three wash bottles and further through the perfusion chambers mounted on three units After five minutes perfusion 0 25 ml 0 01 N $Ba(OH)_2$ (with thymolphtalein as indicator) was pipetted into the central chambers of the units by means of a 1 ml KROGH syringe The perfusion was then stopped and the holes in the lids immediately closed with glass stoppers The CONWAY units were weighed to the nearest tenth of a mg After equilibration the samples were quickly pipetted into the outer chambers of the units with immediate replacement of the glass stoppers and the units weighed again Of the three units perfused simultaneously one was used for blank titration Sixty minutes were allowed for diffusion After that time the titration was carried out with 0 02 N HCl (Titrisol MERCK) in a stream of carbon dioxide free nitrogen gas The normality of the acid as well as of other acids used was checked by titration with acid phtalate (N B S) as the acidimetric standard

The amount of carbon dioxide titrated was expressed as $\mu M/g$ and calculated according to the formula

$$CO_2\ (\mu M/g) = \frac{0\ 02\ (T_b - T\ )}{2\ \ W} \tag{8}$$

where T_b and T are μl 0 02 N acid titrated for blank and sample respectively and W is the weight of the sample in grams

The solubility coefficient (γ) was calculated according to the formula

$$\gamma = \frac{760\ \ CO_2\ \ 22\ 26}{P_{CO_2}\ \ 1000} \tag{9}$$

In calculating γ the carbon dioxide concentration was expressed as μM per g of water in the solution The amount of water in the tissue was measured by drying pieces

Table I *Solubility coefficient (ml CO_2/g) of carbon dioxide in water 0 16 M NaCl and cerebrospinal fluid at 37 5 C $\bar{X}$ = mean value of the group N = number of determinations σ_m = standard deviation of mean In calculating the solubility coefficient for water the depressive effect of the added hydrochloric acid was corrected for by adding 0 0003*

Solution	$\bar{X}$	Range	N	σ_m
Lactic acid 0 1 N	0.5510	0.5468–0.5584	8	—
HCl 0 01 N	0 5518	0.5451–0 5592	19	0 0010
Water acidified	0 5518	—	27	0 0011
Water not acidified	0.5607	0.5501–0.5681	20	0 0010
NaCl 0 16 M	0.5306	0 5253–0.5377	12	0 0015
CSF	0 5296	0.5220–0.5358	23	0 0010

of tissue overnight at 110 C and determining the loss of weight This is the usual procedure for obtaining the amount of water in the tissue (see *e g* VAN SLYKE *et al* 1928)

In accordance with VAN SLYKE *et al* (1928) it was assumed that the undissociated lactic acid as well as the hydrogen ions added to the homogenates did not influence the carbon dioxide solubility The effect of the chloride ions added and lactate ions formed was however allowed for by using the correction factors determined by the above workers The chloride ion concentration was taken as that of the hydrochloric acid corrected for the dilution in the homogenates The concentration of lactate ions was calculated from the HENDERSON HASSELBALCH equation using a pK′ for lactic of 3 74

The pH of the homogenates was determined at 37 5 C with the micromethod of SIGGAARD ANDERSEN ENGEL, JORGENSEN and ASTRUP (1960) All pH values were to the 0 05 M phtalate phosphate and borate buffers according to the standards of N B S (BATES 1954) (for interpolated values at different temperatures see MATTOCK 1958) The phtalate and borate buffers were used to check the linearity and the amplification while the phosphate served as primary standard for all individual measurements The buffer solutions were prepared from freshly boiled distilled water and corresponding N B S salts

Results

The solubility of carbon dioxide in water Prior to the solubility measurements the titration method was tested on freshly prepared carbonate solutions. Eighteen determinations on a solution containing 10 µmoles Na_2CO_3/g (MERCK p a) gave as a result 9 99 ± 0 02 µmoles/g (mean ± S D of mean) The procedure used to determine the solubility was then tested by measurements of the solubility coefficient for carbon dioxide in water at 37.5 C using solutions of 0 1 N lactic and 0 01 N hydrochloric acid. The results of these measurements as well as of the measurements on 0 16 M NaCl and on cerebrospinal fluid, are shown in Table I It is seen that there is no significant difference between the two kinds of acidified water solutions The mean value of all measurements on water is 0 5518 (ml CO_2/g water) If this value is

corrected to 38° C assuming a 2 per cent change per degree (BARTELS and WRBITZKY 1960) 0.546 is obtained. The latter value should be compared with the coefficients 0.547 (VAN SLYKE *et al* 1928) and 0.546 (BARTELS and WRBITZKY 1960) earlier determined at 38° C. There is thus an excellent agreement between the three sets of determinations The divergent values obtained by other workers however made it desirable to determine the solubility coefficient also in water which had not been acidified previously (see Discussion) It is seen in Table I that the coefficient thus obtained is almost 2 per cent higher than that obtained on the acid solutions

The solubility of carbon dioxide in 0.16 M NaCl and in cerebrospinal fluid. The solubility of carbon dioxide in CSF has been assumed to be the same as in a salt solution of the same ionic strength (*cf* ALEXANDER CELFAND and LAMBERTSEN 1961) To test this assumption measurements were made on 0.16 M NaCl solutions and on acidified CSF (Table I) The coefficient obtained at 37.5° C on the 0.16 M NaCl solutions 0.5306 (ml CO_2/g solution) is in good agreement with the value of 0.527 (ml CO_2 ml solution) at 38° C which can be derived from the work of VAN SLYKE *et al* (1928 Fig 2 p 776) It is also seen that an almost identical solubility coefficient (0.5296 ml CO_2/g solution) was obtained on the CSF a fact which strengthens the above assumption.

The solubility of carbon dioxide in cortical tissue The titration method used measures the amount of carbon dioxide dissolved in the sample at the time of withdrawal from the tonometer and any carbon dioxide which could possibly be formed during the time allowed for diffusion. In order to evaluate this source of error 18 determinations were made on different homogenates equilibrated with carbon dioxide free nitrogen gas In 11 of these no carbon dioxide was evolved the mean value of all 18 determinations being 0.03 μmoles/g homogenate. The carbon dioxide produced should thus be an insignificant fraction of that dissolved when the homogenates were equilibrated with pure carbon dioxide At low carbon dioxide concentrations the above figure will however form a significant part of the total amount of gas evolved (about 10 per cent at 1 per cent carbon dioxide)

Seventeen experiments were carried out on the homogenates The α and the α values obtained were plotted against per cent by weight of cortical tissue in the homogenates (Fig 2) Each individual value in the figure is the mean of two to four determinations (usually two double samples each with its own blank) The per cent by weight of brain tissue in the homogenates varied between 5 and 45 per cent Homogenates more concentrated than that could not be investigated as they did not flow freely enough to permit equilibration In all homogenates pH was below 3.80 In a given homogenate pH variations did not seem to have any influence upon the results as judged from the coefficients obtained with identical homogenates except for the acid being 0.2 N It is seen in the figure that there is a linear relation between solubility coef-

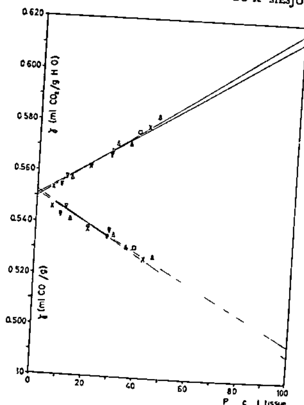


Fig 2 The solubility coefficient (ml CO_2/g) of carbon dioxide in cerebral cortical homogenates at 37.5 C. The individual symbols denote experiments on different animals Note linear relationship between solubility coefficient and tissue concentration in the homogenates also the intersection of the regression lines with the zero per cent line at the point previously determined for water (see text) The extrapolation of the regression lines to the 100 per cent line gives the solubility coefficient for undiluted tissue.

and tissue concentration in the homogenates The solubility coefficient for undiluted tissue was found by extrapolation of the regression lines assuming linearity also at higher cell concentrations The slope of the regression lines and the intersection with the zero and the 100 per cent lines were found by applying the formula of least squares to the homogenate values The values thus found for the γ and the γ of undiluted cells were 0.492 (ml CO_2/g) and 0.611 (ml CO_2/g of water) respectively The analysis further showed that both regression lines intersected the zero per cent line at 0.551 a value that comes very close to that actually determined for water (0.5518)

The solubility of carbon dioxide at low carbon dioxide tensions The possibility of an *adsorption* of carbon dioxide to structures in the tissue must be borne in mind Thus there is some indication that carbon dioxide may be adsorbed to particles in collodial solutions (FINDLEY and WILLIAMS 1913) although the lack of information about pH in these studies excludes any definite statement. Since adsorption of carbon dioxide should manifest itself by a deviation from HENRY's law, determinations of the solubility coefficient were performed at low carbon dioxide tensions At 25 and 15 per cent carbon dioxide in the equilibration gas no deviations were found from the coefficients obtained with pure carbon dioxide At still lower concentrations however, there was

Table II Solubility of carbon dioxide at low carbon dioxide tensions γ was calculated according to equation (9) Δ CO_2 is the difference between the amount of carbon dioxide dissolved in the above experiments and that dissolved when the equilibration gas was pure carbon dioxide calculated per a mm Hg a mm Hg is the carbon dioxide tensions in the above experiments

P_{CO_2} mm Hg	Per cent homog	γ ml CO_2/g	Δ CO_2 μM/g
9 1	13 1	0 587	0 02
9 1	36 6	0.560	0 02
36 6	15.2	0 542	0 00
36 6	31 9	0 554	0 05
36 6	36.3	0 551	0 05
66 0	19.5	0 541	0 01
66 0	6 8	0 539	0 04

a progressive increase in the coefficients obtained (Table II) This increase was suggestive of an adsorption If however the differences between the amount of carbon dioxide evolved at the lower tensions and that obtained with pure carbon dioxide for the same tension were plotted the Δ CO_2 values in column 4 were obtained These differences were evidently not greater than that they could be explained by carbon dioxide production in the homogenates (see above) The results thus gave no indication of an adsorption of carbon dioxide in the homogenates but showed that within the experimental error of the present method and at the actual pH values the solubility of carbon dioxide followed HENRY s law

Calculation of carbonic acid concentration in the homogenates The solubility coefficient (γ) derived from the present work can be used to calculate the amount of carbonic acid (by convention the total amount of carbon dioxide dissolved) in different cortical tissue homogenates at any carbon dioxide tension To do this one has only to draw a straight line between a zero point representing the solubility in the liquid used for homogenization and a 100 per cent point representing the solubility in undiluted tissue A simple interpolation will then give the solubility in the homogenate at any tissue concentration If the amount of carbon dioxide is expressed as μM/g it will be given by the relation

$$CO_2\ (\mu M/g/mm\ Hg) = P_{CO}\ S \quad (10)$$

where S is the solubility factor given by the equation

$$S = \frac{\gamma\ 1000}{760\ \ 22\ 26}$$

The S values for the homogenates using either water or 0 16 M homogenization fluid are shown in Fig 3 If another homog

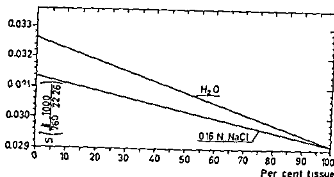


Fig 3 Carbon dioxide solubility factor for interconversion of carbon dioxide tension (mm Hg) to carbon dioxide concentration (μM/g) The lines given are applicable to homogenates prepared with water and 0 16 M NaCl respectively

is used a new line can be constructed, by inserting the solubility factor for the particular fluid as zero point In finding the solubility coefficient for carbon dioxide in the fluid used advantage can be taken of the fact that the effects of the added ions upon the solubility are additive (VAN SLYKE *et al* 1928 MARKHAM and KOBE 1941)

Discussion

The solubility of carbon dioxide in water The validity of the titration method used in the present investigation for the determination of the carbon dioxide content was confirmed by the measurements on the carbonate solutions Likewise, the close similarity between the present solubility coefficient for rbon dioxide in water and two such coefficients determined earlier (VAN YKE *et al* 1928, BARTELS and WRBITZKY 1960) at first indicated that the cedure followed in determining the coefficient was a valid one A study of the literature concerning carbon dioxide solubility did reveal however that the above coefficients were lower than several others reported Although the discrepancies were commented on sometimes no explanation was put forward to account for them In order to find the explanation a comparison was made between coefficients determined at 25 and 38 C The OSTWALD coefficients (λ) and the HENRY's law constants ($Q = m_{CO_2}$/atm) were converted into BUNSEN coefficients (α) by applying the formulae (at 25 C)

$$\alpha = \frac{273}{298}\lambda \qquad \alpha = 22\,26\ Q\ d$$

where d is the density of water At 38 C the comparison was made between the KUENEN coefficient (γ) after correction for the density of the solutions used (Table III) The volume of 1 mg molecule of CO_2 at 0 C and 760 mm Hg was taken as 22 26 ml

It is seen in the table that at 25 C the majority of coefficients approximates to 0 755 while one (0 765 HARNED and DAVIS 1943) is considerably higher and another (0 743 BARTELS and WRBITZKY 1960) considerably lower than all the others The KUENEN coefficient obtained by the latter workers at 38 C

Table III Comparison between solubility coefficients reported. λ = OSTWALD coefficient (ml CO_2/ml, gas volume measured at actual temperature) α = BUNSEN coefficient (ml CO_2/ml gas volume reduced to standard conditions) γ = KUENEN coefficient (ml CO_2/g gas volume reduced to standard conditions) The different coefficients reported were interconverted by the suitable equations (see text) The volume of 1 mg molecule CO_2 was assumed to be 22 26 ml

Investigator	λ 25	α 25	γ 38
BOHR 1899			
JUST 1901	0 826	0 757	0 559
GEFFCKEN 1904	0 876	0 756	—
FINDLEY-CREIGHTON 1910	0 826	0 756	—
FINDLEY SHEN 1912	0 817	0 749	—
FINDLEY WILLIAMS 1913	0 825	0 756	—
KUNERTH 1922	0 816	0 748	—
v SLYKE *et al.* 1928	0 818	0 749	—
MORGAN PYNE 1930	—	—	0 547
SHEDLOVSKY MACINNES 1935	0 827	0 757	—
KOBE-WILLIAMS 1935	0 819	0 751	—
v KISS *et al* 1937	—	0 754	—
CURRY HAZELTON 1938	0 819	0 750	—
MARKHAM KOBE 1941	0 821	0 [illegible]52	—
HARNED-DAVIS 1943	—	0 757	—
BARTELS-WRBITZKY 1960	—	0 [illegible]65	0 556
PRESENT WORK acidified	—	0 743	0.546
PRESENT WORK, not acidified	—	—	0.546
	—	—	0.555

(0 746) is however in close agreement with the coefficients obtained by VAN SLYKE *et al* (1928) and by the author It is also clear that there is a marked difference between these three coefficients and all others when a comparison is made with the other coefficients measured at 38 C

The cause of the discrepancies between the solubility coefficients reported seems to reside in the base content of the water used Thus the three low coefficients referred to above were all obtained when acidified water was used whereas in none of the other papers was a statement made about any acidification The values within the latter group also spread considerably a fact that is compatible with the presence of variable amounts of base in the waters investigated The explanation proposed is strengthened by the present result on non acidified water The KUENEN coefficient thus obtained is in fair agreement with the values obtained at 38 C by HARNED and DAVIS (1943) and by BOHR (1899) The problem of the true solubility of carbon dioxide thus seems to involve a question of terminology It is clear however that if a distinction is to be made between true solubility and chemical binding the solubility coefficient should be measured on water which does not possess the ability to bind carbon dioxide in the form of bicarbonate The same argument must hold for salt solutions

The solubility of carbon dioxide in cortical tissue homogenates The method used to measure the solubility of carbon dioxide in the homogenates does include errors resulting from any carbon dioxide produced by the tissue and errors resulting from the handling of the frozen tissue The control experiments referred to above did not indicate any carbon dioxide production of an order sufficient to influence the results As for the second source of error, it must be concluded that the intersection of the regression lines with the zero concentration line at the value previously determined for water should exclude any large systematic error The spread of the individual homogenate values also indicates that the solubility coefficient derived for undiluted tissue is valid in the second decimal place provided, of course, that a straight relationship between solubility coefficient and tissue concentration can be assumed to exist also at high tissue concentrations The latter assumption cannot at present be proved experimentally As remarked by VAN SLYKE *et al* (1928) it appears improbable however that the effect of the electrolytes and the solids of the tissue upon the solubility should be other than linear Another assumption, which cannot be subjected to experimental proof, concerns the effect of the replacement of the buffer anions of the tissue upon acidification There is thus no way to decide whether the undissociated buffer acids influence the solubility to another degree than do the buffer anions although it is clear that the difference if any, should be small under the present conditions (*cf* VAN SLYKE *et al* 1928)

The experiments on the homogenates clearly show that the solubility of dioxide per gram homogenate decreases with increasing amounts of tissue constituents The solubility per gram of water in the solutions however, shows an increase with increasing cell concentration The latter finding indicates that apart from water some other tissue constituent (or constituents) acts as a solvent for carbon dioxide and it is tempting to ascribe the solving capacity to the lipids of the tissue A positive statement cannot however be made on this point Thus the cerebral cortex contains only negligible amounts of neutral fat (*cf* McILWAIN 1959) and nothing is known about the influence of the special lipids of cortical tissue upon the solubility of carbon dioxide Further it cannot be excluded that other cell constituents like proteins act as solvents for carbon dioxide

It is debatable if measurements on fat free tissue homogenates (NICHOLS 1958) do give any useful information on the solubility of carbon dioxide in the brain Thus a coefficient obtained from such experiments cannot be used to distinguish between the amount of carbon dioxide dissolved and that bound respectively Moreover the application of a coefficient thus obtained to acid base calculations will be of very limited value as long as the pK_1 of carbonic acid is not determined on the same system. Finally such a determination would still be adventurous partly because the lipids undoubtedly contribute to the carbon dioxide buffering partly because the lipid extraction is apt to

change the ionic strength of the system since inorganic salts are removed (FOLCH, LEES and SLOANE STANLEY 1957)

The main value of the present experiments is the determination of a coefficient (γ) which makes it possible to distinguish between the amount of carbon dioxide dissolved and that bound as bicarbonate ions respectively Thus if the carbon dioxide tension as well as the total carbon dioxide content are known the bicarbonate concentration of the tissue is easily calculated (*cf* equation (1)) The γ coefficient has no useful meaning other than that it demonstrates the capacity of the tissue constituents to dissolve carbon dioxide None of the coefficients, however is a good measure of the S factor in the denominator in equation (1) since this factor should denote the carbonic acid concentration in the water phase of the system under study (*cf* SIESJO 1962 c)

In the present work no attempts were made to separate the tissue into extra and intra cellular compartments The present controversary about the size and even the existence of an extracellular space in the brain gives no rational basis for such a procedure Moreover, it can be shown theoretically that it is highly improbable that a large part of the bicarbonate of the tissue is confined to the conventional chloride space (SIESJO 1962 b) It is felt that until further knowledge is available concentrations are preferably related to the tissue as a whole Such a point of view does not involve any greater draw back with regard to the present results as their principle value should be the possibility to distinguish between the carbon dioxide dissolved and that bound So applied the present extrapolated coefficient might even be assumed to be valid for intact tissue

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Acta physiol scand. 1962 55 342—352

From the Institute of Medical Physiology University of Copenhagen Denmark

The Renal Excretion of Lipoid Soluble Substances as Exemplified by the Excretion of Ethanol

By

CHRISTIAN CRONE

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Abstract

CRONE C *The renal excretion of lipoid soluble substances as exemplified by the excretion of ethanol* Acta physiol scand 1962 55 342—352 — The present communication deals with the excretion pattern of ethanol an organic substance for which the kidney is a very poor organ of excretion The purpose of testing this substance was to see whether the general laws relating chemical constitution and ability to penetrate cellular membranes as formulated by COLLANDER and BARLUND are applicable to the renal tubular cells The findings were in accordance with that general rule Contrary to what is usually found for passively transferred substances with higher clearances, the U/P ratios were found to be lowest at low rates or urine flow The U/P ratios varied between 0 9—1 1 over a wide range of rates of urine flow Further it was shown that for ethanol the renal papilla only very slowly attains equilibrium with the arterial plasma and as ethanol in the final urine is probably in equilibrium with the interstitial tissue of the papilla this fact stresses the absolute necessity of maintaining a constant concentration of ethanol in the arterial blood in experiments on the renal treatment of this substance Where easily diffusible substances are concerned the renal papilla behaves to some extent as an autonomous part of the kidney

NICHOLES and HERRIN's investigations (1941—42) on the renal excretion of different non electrolytes urea thiourea and derivatives of thiourea showed that the clearance of these substances diminished as the lipoid water partition coefficient rose This important observation suggested that the passive back diffusion of non electrolytes from the tubular lumen to the interstitial fluid takes place in accordance with the general biological law for the passage of non-electrolytes across cell membranes formulated by OVERTON (1902) and

later by COLLANDER and BARLUND (1933) They showed that the ability to pass biological membranes by diffusion is dependent on the tendency of the molecule to form hydrogen bonds with the water molecules or as they stated the polarity of the molecule is the most important single physico-chemical characteristic determining the rate of passage[1] Many investigations on the renal excretion of typically polar substances (polyvalent alcohols and carbohydrates) have been published, but the excretion pattern of less polar or non polar substances has hardly been investigated A study of the renal excretion of ethanol may therefore be of some interest

It is known that only small amounts of ethanol about 1—2 % of the original dose are eliminated by the kidneys This indicates the absence of any marked ability of the renal tubules to concentrate ethanol and so the substance may be grouped in accordance with the Collander Barlund scheme However, it is not known whether or not the renal tubules can concentrate ethanol to a small extent or if under steady state conditions the ethanol concentration in the urine is identical with that of the water phase of the plasma In the present series of experiments the U/P_w ratio[2] for ethanol has been determined over a wide range of urine flow rates Earlier experiments on renal excretion of ethanol have only been performed under conditions in which the kidneys were producing a dilute urine as ethanol blocks the release of vasopressin from the neurohypophysis This report includes observations made at very low rates of flow established by the continuous infusion of antidiuretic hormone

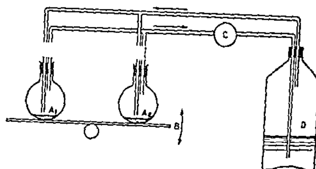
As the ethanol concentration in the urine is close to the concentration in the water it is reasonable to assume that it is the concentration of ethanol in the renal interstitial tissue that determines the concentration in the urine It has become apparent in recent years that the kidney may be regarded as consisting of two separate parts from the point of view of blood perfusion (cortex and medulla) because there is a ratio of 20 1 between the cortical and medullary blood flows (KRAMER THURAU and DEETJEN 1960) In consequence the medullary parts of the kidney will only reflect changes in the arterial concentration of infused test substances relatively slowly and the counter current exchange mechanisms of the medullary capillaries will further delay the equilibration of the arterial plasma and the inner parts of the kidney especially in the papillae These considerations together with the expected equilibration of ethanol between the collecting duct urine and the medullary interstitial fluid stress the importance of maintaining a constant blood level of ethanol in investigations on the U/P ratio of this substance

Experiments were made to find how important it is to maintain a constant level of blood ethanol Observations were made of the changes in concen

[1] The term polarity is used h re to describe the p ope ty which d pends on the polarizable groups p esent in the molecule rather than on th magnitud of the dipole moment.

[2] P_w signifies the concentration in the water phase of the plasma, calculated as the plasma concentration × 1 05

Fig 1 Apparatus for the equilibration of plasma and urine with ethanol The vessel D contains an aqueous solution of ethanol Vapour from this is circulated through the apparatus by the pump C The flasks A_1 and A_2 contain urine and plasma respectively and are mounted on a rocking table B



tration of ethanol in the urine which follow the rapid establishment and subsequent maintenance of an approximately constant level of plasma ethanol From these results the rate at which the renal papilla comes into equilibrium with the arterial blood has been estimated

Substances with a considerable vapour pressure at body temperature do not necessarily have the same concentration in different tissues even if tension differences do not exist In other words the proportionality constant (k=conc/tension) which relates tension to concentration is not necessarily the same for plasma as for urine If it were greater for plasma than for urine it might be expected that the ethanol concentration in urine should be lower than that in plasma even if an equilibrium had been established between the two phases

As it was indeed found that in some cases the U/P_w ratio was less than 1, in vitro experiments were performed to determine the ethanol concentration in plasma and urine after the two substances had been equilibrated with ethanol at the same tension

Material and Methods

1 *Determination of the U/P ratio for ethanol* The experiments were performed on female mongrel dogs (weight 13—23 kg) In the majority of the experiments the animals were anesthetized with Nembutal© 25 mg/kg body weight In a few cases experiments were carried out on unanesthetized trained animals because it was difficult to obtain high urine flows in anesthetized animals A priming dose of ethanol was given through a gastric tube after suitable dilution with water The blood concentrations of ethanol were usually between 100—200 mg/100 ml Constant levels of ethanol in the blood were obtained by continuous infusion of ethanol in a 0 9 per cent solution of NaCl At least 1½ hour elapsed between the injection of the priming dose and the start of the experiment In experiments at high urine flows the animals were loaded with water (about 3 % of the body weight) in experiments at low urine flows Pitressin was administered by continuous infusion of 10—40 mU/min Blood was sampled from the femoral artery Urine was collected through an indwelling catheter introduced into the bladder through the urethra It was assured that no ethanol was lost by evaporation during the collection period normally 15—20 min

2 *Experiments to determine the ethanol concentration in plasma and urine at equal tensions* The experimental procedure may be seen from Fig 1 The flasks (A) contain plasma

and ethanol resp B is a rocking table securing convection in the fluids C is a one way pump driving air through the system and thus circulating the ethanol vapour from the reservoir D containing an ethanol solution ca 400 mg/100 ml The duration of the experiments was 45 min

3 *Determination of the time required for equilibration with ethanol of arterial plasma and the tissue in the renal papilla* Ideally these experiments require a constant concentration of ethanol in the arterial blood from the very beginning of the experiment By means of a diminishing infusion rate it was attempted to supply ethanol at a rate corresponding to the loss from the blood during the period of distribution in the body water A diminishing infusion rate was obtained by means of a logarithmically declining pressure head the latter being provided from a compressed air reservoir from which the air escaped to the atmosphere through a constant resistance (capillary tube) Since upon establishing a constant plasma ethanol level the rate of loss of ethanol from the plasma does not fall with time as a single exponential function the successive use of two different resistance tubes was found necessary to obtain reasonably constant plasma ethanol levels

The time relation of the rise in concentration of ethanol in the urine was followed by sampling urine over periods of a few minutes duration In order to avoid delay in the passage through the lower urinary tract the urine was collected through a short, thin polyethylene catheter inserted in the renal pelvis

4 *Determination of the loss of ethanol by diffusion in the lower urinary tract* In these experiments a polyethylene catheter was inserted into the ureter just beneath the pelvis A solution of ethanol was infused through the catheter and the perfusate was collected through another catheter in the bladder By varying the infusion rate the loss by diffusion could be determined at different rates of volume flow The amount of ethanol diffusion through the ureter and the bladder wall was investigated in other experiments in which the urine from one kidney was sampled through a catheter in the pelvis and that produced by the other kidney from a catheter in the bladder

Analytical technique Ethanol was determined enzymatically by the method described by LUNDQUIST (1959) In the presence of alcohol dehydrogenase ethanol is oxidized with simultaneous reduction of DPN to DPNH The rise of density at 3 400 Å is proportional to the DPNH formed and thus the amount of ethanol oxidized By adding ethanol to plasma and urine to a concentration of about 100 mg/100 ml the following recoveries were found for plasma and urine resp 101 5 % (coefficient of variation 0 4 n = 11) and 100 0 (coefficient of variation 1 0 % n = 11)

In several cases it was observed that urine gave blank values which were higher than those for pure water This difference in the blank values varied from sample to sample and it was therefore found to be necessary to determine the optical density of the urine alone (without the addition of DPN and enzyme) after dilution in a volume of water similar to that used in the enzymatic procedure Failure to make this correction can lead to erroneously high values for the urine concentration Absorption at 3 400 Å as a result of the presence of excretion products of Nembutal was eliminated by this procedure

In a few instances another source of error was detected ethanol free urine that had been allowed to stand in the laboratory for a day might show DPN reducing activity as if ethanol had been present By treating the urine with perchloric acid immediately after the sampling (as in the deproteinisation of plasma samples) this source of error could be eliminated The nature of the phenomenon was not further elucidated

In a control animal anesthetized with Nembutal no ethanol was found in the urine in an experiment lasting 3—4 hours

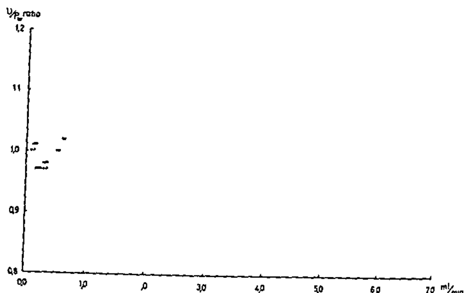


Fig 2 U/P_w-ratio s for ethanol at varying rates of urine flow Ordinates U/P_w-ratio Abscissae urine flow ml/min (For the meaning of U/P_w-ratio see text)

Calculation of the U/P_w-ratio As stated in the foot note a calculated concentration in the plasma water phase was used instead of the concentration in the plasma itself The calculation of the ethanol in the plasma water was performed by means of a constant correction factor 1 05 Assuming a mean protein concentration in plasma of 7 g/100 ml and a partial specific volume of the proteins of 0 7 the volume occupied by water would be 95 % of the total volume of the plasma

Results

1 *U/P_w ratio of ethanol* 20 experiments were performed on 8 dogs The urine flow varied between the limits 0 05 ml/min and 6 8 ml/min The results are given in Fig 2 It can be seen that the U/P_w ratio is always close to 1

At low rates of urine flow the ratio tends to be somewhat lower than 1 while at flow rates higher than 3 ml/min it tends to stabilize at 1 1 The results in one dog deviated from this pattern in 2 experiments on this animal U/P_w ratio s of 1 05 and 1 16 were found at diureses as low as 0 2—1 5 ml/min (these results are not shown on the figure)

Observations at low urine flows were made in experiments where the low flow rates were obtained by infusion of pitressin and also in experiments where the low flow rate was spontaneous No difference could be detected as a result of these two different experimental conditions

For ethanol the pattern of excretion has thus been found to differ from that which is usual for other substances like urea which are assumed to be subject to only passive transfers in the kidneys in as much as the smallest degree of concentration was found at the lowest urine flow

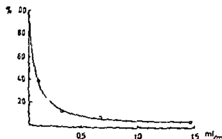


Fig 3 Ethanol loss from the lumen of the ureter at varying rates of flow through the ureter Ordinates Per cent loss Abscissae Flow rate of perfusion fluid

From medico-legal investigations it is known that one can find rather high U/P ratios of ethanol when the concentration of ethanol in the bladder urine (formed during an ill defined period of time) is compared with the concentration in the plasma drawn at the time of urine collection This can probably be explained by the fact that most of the urine was secreted over a period during which the blood ethanol concentration was higher than at the time of collection of the urine sample One experiment in the present series was performed on falling plasma concentrations and the urine was permitted to stay in the bladder in this case U/P_w ratios of 1 34 1 40 and 1 66 were found (the urine remained in the bladder for one hour in the first two cases and for 2 hours in the last case)

2 *U/P_w ratio of ethanol as determined on urine from the pelvis and from the bladder* In order to investigate whether the low U/P_w ratios found at low diureses were due to equilibration between urine and plasma during the passage through the lower urinary tract experiments were performed in which the urine from one kidney was collected from the pelvis and that formed by the other from the bladder In 2 experiments of this type (with 11 collection periods) it was found that the U/P_w ratio in the pelvis urine was 1.03 ± 0.04 (S D $n = 11$) and in the bladder urine 0.98 ± 0.04 (S D $n = 11$) The experiments showed that the low U/P_w ratio is already present in the newly formed urine and that the U/P_w ratio is reduced somewhat during the passage through the ureter as the difference between the two figures is significant ($P < 0.02$)

3 *Exchange of ethanol between urine and interstitial fluid in the lower urinary tract* The magnitude of the diffusion loss of ethanol in the lower urinary tract was investigated in experiments where a large concentration gradient was established between the fluid in the ureter and the blood In an animal not containing ethanol the ureter was perfused with a solution of ethanol (166 mg/100 ml) and the perfusion fluid was sampled through an indwelling catheter in the bladder By varying the perfusion rate the diffusion loss could be determined at different diureses The results are given in Fig 3 which shows that at volume flows greater than 1 ml/min the diffusion loss is insignificant while it is of importance at very low diureses

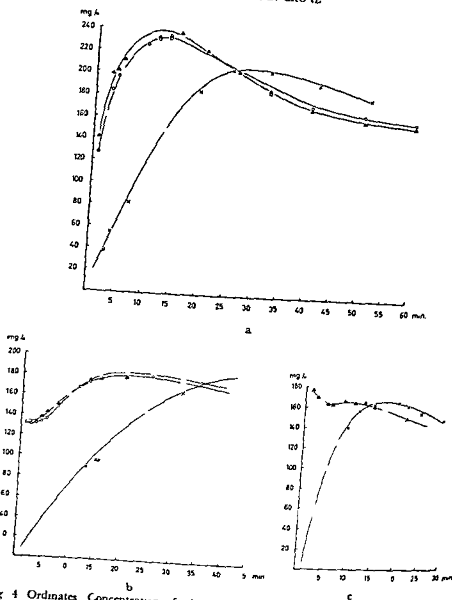


Fig 4 Ordinates Concentration of ethanol in the renal artery and vein and in the urine collected from the renal pelvis Abscissae Time in minutes from the start of the ethanol infusion The concentrations in the blood are given as the concentrations in the water phase ▲——▲ arterial concentration o——o venous concentration x——x concentration in pelvic urine The three figures show the results from three different experiments.

4 *The time necessary for equilibration of ethanol between the arterial blood and the renal papilla* These experiments were based on the assumption that the concentration of ethanol in the newly formed urine sampled from the renal pelvis represent the ethanol concentration in the interstitial fluid of the innermost part of the kidney By establishing a reasonably constant ethanol level

in the blood from the start of the experiments it should be possible to get an impression of the rate at which the renal papilla equilibrates with the arterial plasma

Three experiments have been performed, the results of which are given in Fig 4 In two of the experiments the rise in concentration in the renal vein was also followed (a catheter was introduced into the vein through the femoral vein) Although it proved difficult to maintain constant plasma levels of ethanol from the start of the experiments they clearly indicate that the tissue in the renal papilla only relatively slowly attains an ethanol concentration similar to that of the arterial plasma perfusing the kidney In these experiments the equilibration times were found to be 13 23 and 36 min The fact that the ethanol concentration in the blood is rising during two of the experiments tends to delay the time for definite equilibration The experiments show however, that the ethanol concentration of the renal effluent blood very rapidly rises towards that of the arterial blood and thus demonstrates the marked difference in perfusion intensity between the cortex and the innermost part of the kidney

5 *The concentration of ethanol in plasma water and in urine at equal tensions* Some in vitro experiments were performed to investigate the possibility that the U/P_w ratio s of less than 1 which had been found in many instances at low rates of urine flow (see Fig 2) were due in fact to a higher solubility of ethanol in plasma than in urine The two liquids were exposed simultaneously to ethanol vapour (see Fig 1) The concentration of ethanol in the plasma water was calculated from the ethanol content per volume plasma (using the correction factor 1 05 see *Methods*) An average ratio (U/P_w) between the absorption coefficients in the two fluids of $0\ 95 \pm 0\ 03$ (S D n = 23) was found thus indicating that plasma is a better solvent for ethanol than urine

Discussion

The reported results all fit into our present conception of the function of the kidneys and only a few comments seem necessary The U/P_w ratios found clearly show the ineffectiveness of the kidney as an organ for the excretion of non polar substances and for those substances whose molecules are dominated by non polar groups (the role of the liver in the elimination of a large group of foreign substances is therefore to convert them into polar forms which can be excreted by the kidneys)

In the analysis of the mechanism of excretion of these types of substances the filtration reabsorption theory is without much relevance It is reasonable to assume that at any point in the passage through the renal tubule ethanol in the lumen equilibrates with the surrounding interstitial fluid If this also takes place in the last part of the nephron the concentration in the urine depends entirely on the concentration in the renal papilla At high and intermediate rates of urine flow U/P_w ratios were found to be a little over unity

This might be interpreted as being due to the back diffusion of ethanol from the collecting ducts lagging behind the reabsorption of water by osmosis at the same site i e a condition to be expected if the latter process produces the gradient required for the former process

The possibility must also be considered that consumption of ethanol by the tissue in the kidney especially in the papilla might be the cause of ethanol U/P_w ratios below 1 PETERSEN and LUNDQUIST (1960) demonstrated the presence of alcohol dehydrogenase in kidney tissue from dogs, but did not make any specific report of the topographical localization in the cortex or medulla

The reported investigations on the tendency of ethanol to equilibrate with the interstitial fluid in the lower urinary tract show that at low rates of urine flow it can be of a considerable order of magnitude This is in accordance with the experiments of GARBY and ULFENDAHL (1956) in which it was shown that D_2O in rabbits exchanges between the fluid in the ureter and the surrounding tissue fluid RENNIE, REEVES and PAPPENHEIMER (1958) investigated the diffusion of oxygen through the ureter in experiments on dogs, where ureter perfusions were performed with solutions of varying oxygen tensions While in the present experiments 50 per cent equilibration first occurred at flow rates as low as 0 06 ml/min these authors found 50 per cent equilibration for oxygen at flow rates 10 times as great as that value This marked tendency of readily diffusable substances (notably water) to display net movements depending on the chemical potential gradient may influence estimates of the concentrating ability of the kidney in a manner that has been partly overlooked in experiments where urine samples were taken after passage through the lower urinary tract

In the literature no information can be found about the rate at which the renal papilla becomes saturated with test substances to the same concentration as the arterial blood The present investigations with ethanol indicate that blood in the renal vein does not reflect conditions in the papilla or inner parts of the kidney and that those regions are charged with ethanol at a much slower rate than the cortex The concentration of ethanol in renal venous blood becomes almost instantaneously the same as that of arterial blood The experiments thus stress that when applied to the kidneys the inert gas technique (KETY 1951) will — in practice — only be capable of measuring the flow through those parts with a high perfusion rate (i e cortex), equilibration of the inner parts of the kidney would not be achieved within an experimental period which would allow the mean arterio-venous concentration difference to be determined with acceptable accuracy

The results of the saturation experiments thus stress that the concentration of ethanol in arterial blood must be held absolutely constant in experiments where the excretion pattern of easily diffusible non polar substances is being investigated. There is a considerable delay in the changes in concentration

in the renal papilla when the arterial concentration varies and under such conditions the urine will therefore contain for some time the test substance in concentrations which are greater or smaller than those present in the arterial blood

Conclusions

Investigations on the renal excretion of ethanol in dogs have shown

1 The relation between the concentration of ethanol in the urine and in the water phase of plasma (U/P_w ratio) is 0 9—1 1 at rates of urine flow varying between 0 05—6 8 ml/min The lowest U/P_w ratio s were found at the lowest urine flows

2 The loss of ethanol by diffusion in the ureter and the bladder is significant at low urine flows By perfusion of the ureter it was shown that 50 per cent equilibration with the surroundings occurred at flow rates of 0 1 ml/min The diffusion loss is insignificant at flow rates greater than 1 ml/min

3 The renal papilla only slowly comes into equilibrium with the arterial plasma. In three experiments the times necessary for saturation were 13 23 and 36 min respectively As the concentration of ethanol in the urine reflects the concentration in the interstitial tissue of the papilla determinations of U/P_w ratio s for this substance are without value if the arterial concentration is not kept absolutely constant during the experiments

4 The concentration of ethanol in the renal vein only reflects the concentration of ethanol in those parts of the kidney with a high perfusion rate *i e* cortex. This means that determinations of the perfusion rate of the kidneys by the inert gas technique (KETY 1951) gives no indication of the perfusion rate of the medulla and papilla

5 Investigations in vitro have shown that at equal tensions of ethanol, more is dissolved in plasma than in urine and the fluid behaves as if the proteins did not occupy a part of the volume This probably explains why U/P_w ratio s lower than one can be found

6 The investigations have supported the view that COLLANDER BARLUND s scheme (1933) can be used to predict the renal clearance of an organic substance when its chemical constitution is known Polar substances having a poor ability to penetrate through cell membranes will be excreted at high clearances whilst non polar or less polar substances will be excreted at low clearances Marked deviations from this general rule are presumably due to enzymatically dependent transport in the tubules

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or limited activity, a long or short working period physical work in the field, or tests e g on the cycle ergometer When assessing physical working capacity for work with large muscle groups during lengthy periods the individual s pulse rate at submaximal work often serves as a starting point An often used method, SJOSTRAND (1947, 1960), is to let the subject work on a bicycle ergometer for 6 min on each of a series of workloads increased step by step until the pulse rate reaches a level of 150—170 beats/min With this method the physical working capacity is indicated to the power level where the pulse rate after inter- or extrapolation, is 170 beats/min (= PWC_{170}) Another method is that of ÅSTRAND, which transforms the pulse frequency for a certain power level to a value of estimated maximal O_2 consumption by using a special nomogram

The question as to which method is the most reliable in an assessment of physical working capacity might be discussed on the basis of, more or less, absolute physiological criteria of true working capacity But the reliability can also be assessed in a purely empirical way so as to form a basis for an operational definition of physical working capacity This does not necessarily mean that an estimated value of the working capacity in a more true sense, maximal O_2 uptake or the power at steady state has a higher degree of reliability or predictive validity than e g the pulse rate at a certain power level

The error of method has often been assessed by re testing individuals This is, perhaps the best way of assessing the reliability if a complex measurement is desired it also includes the variation of results for a certain period of time But if we want to estimate the reliability in a more restricted sense, i e the technical accuracy of the test instrument, the individual inconstancy between the tests should not be allowed to have any effect It seems to be of interest, therefore to attempt an estimate of the reliability in this more restricted sense for such a physiologically important measurement as e g, the pulse and breathing frequency during a work test

A study is also made of the degree of validity of the work test i e how it correlates with an independent external criterion of the physical working capacity The criterion here represents results from a performance which besides a special technical ability is greatly dependent upon the individual s physical working capacity viz skiing race results

In this investigation an attempt has been made to study the reliability and validity of some different measurements of importance for the physical working capacity

Material

In 1958 at a regiment in Umeå DAHLSTROM and HAMBRAEUS tested the working capacity of practically all 20-year old forest workers on the cycle ergometer during the period of their military training The tests were performed in the summer of 1957

immediately after drafting (first test) and were then repeated before the end of the training in the spring of 1958 (re-test) Out of the tested 78 recruits 42 had taken part in a 70 mile (30 km) skiing race about one month before the second test (Some of the recruits on fatigue duty or belonging to Test Group 1 of the enrolment test including debile individuals were excluded) The group of 42 recruits formed a fairly representative sample made up of individuals characterized inter alia by height with a normal dispersion around M = 174 0 ± 0 8 cm body weight of M = 68 2 ± 1 0 kg and average physical working capacity from the 1st and 2nd tests PWC M = 1 280 ± 35 kpm/min. The general intelligence test at the time of enrolment gave surprisingly normal results However the preponderance was somewhat below 5 in the 9-graded standard scale and no representatives of Group 9 including the most intelligent individuals were found

Method

The bicycle ergometer test was carried out with successively increased power levels and for a period of 6 min for each level 600 900 1 200 etc kpm/min The pulse frequency was determined after working periods of 2 4 and 6 min and the breathing frequency after 3 and 5 min for each level The pulse rates were obtained from the ECG-record and each value was based on 3 to 4 sec work. The breathing frequency was recorded by counting the number of expiratory puffs on the outstretched hand of the leader of the experiment.

In order to obtain a measure of the intra test consistency a reliability coefficient was assessed by correlating the pulse rates with each other after 2 4 and 6-minute periods of work Two intra test measurements of the power at the pulse rate of 170 beats/min (PWC_{170}) were also obtained by using two pulse rate figures one from 4 and the other from 6 min work for inter or extrapolation In order to obtain a reliability coefficient based on measurements from the same test occasion in accordance with Åstrand s method of calculation the maximal O_2 uptake estimated from pulse rates from the first power level (within the nomogram (for the majority of experimental subjects 600 kpm/min but for a few 900 kpm/min) were correlated with the corresponding results from the second power level (900 kpm/min with a few values from 1,200 kpm/min)

As a supplement to the assessment of the intra test reliability several re test correlations have also been calculated from most of the measurements in both tests As a criterion of condition for test validation results from a 20 mile (30 km) skiing race were chosen. The results consisting of time values varied approximately between 2 and 4 hours and revealed a positively somewhat skew distribution These values were transferred to a 9 graded standard scale A rough measurement of the reliability of the criterion was obtained as 15 of the 42 men also took part in a subsequent 10 mile (15 km) skiing race

Results

In Table 1 the mean values dispersions and ranges for different variables are given The pulse rates and breathing frequencies reveal fairly symmetrical distributions and agree with the averages between both tests A decrease of the physical working capacity from the 1st to the 2nd tests was however obtained The working capacity calculated according to PWC_{170} is — M_1 = 1 308 7 and M_2 = 1 249 5 kpm/min which denotes a significant difference

Table I *Mean standard deviation (S D) and range (d) for different variables at the first (I) and second (II) tests*

N = 42

			M	S D	d
		Chest width I test	94 4	4 5	87– 107
		Body weight M (I + II)	68.2	6 7	54– 81.5
		Height I test	174 0	4 9	161– 185
600	I	Pulse frequency 4	118 6	11 5	95– 139
		Pulse frequency 6	120 9	11 4	99– 147
		Breathing frequency M (3 + 5)	22 2	4 1	11– 31
600	II	Pulse frequency 4	116 9	12.8	9[illegible]– 147
		Pulse frequency 6	120 3	13 0	9[illegible]– 147
		Breathing frequency M (3 + 5)	21 1	3 7	10– [illegible]6
900	I	Pulse frequency 4	140 7	13 9	115– 165
		Pulse frequency 6	142 1	14 2	119– 1[illegible]0
		Breathing frequency (M 3 + 5)	25 1	5 1	12– 33
900	II	Pulse frequency 4	139 6	15 6	107– 177
		Pulse frequency 6	142 8	16 6	109– 180
		Breathing frequency M (3 + 5)	23 7	4 4	10– 33
I		PWC_1 4	1 334	245	1 000–1,6[illegible]0
		PWC 6	1 309	237	[illegible]00–1 710
		O_2 upt., Åstr M (1st + 2nd level)	3 5	0 6	2.55–4 [illegible]0
II		PWC 4	1,295	213	865–1 [illegible]00
		PWC 6	1 2[illegible]0	210	835–1,580
		O_2-upt., Åstr M (1st + 2nd level)	3 5	0 6	2.38–5.[illegible]0

($P < 0.01$ at the t test) No difference between the tests in estimated O_2 uptake according to Åstrand s method of calculation is however obtained in accordance with the fact that there is no difference in pulse rate at the 600 or 900 power levels But as the $PWC_{1\,0}$ values are founded on pulse rates from the whole power range the obtained difference must be considered an interesting fact

Many product moment correlation coefficients have been calculated. It will be seen from the correlation matrix Table II that the correlation between the pulse rates is good The highest intra test correlations are found between the pulse rates from 4 to 6 min to be $r = 0.97$ and $r = 0.98$ at the 1st and 2nd tests for 900 kpm/min The corresponding correlations for the 600 kpm min level are $r = 0.90$ and $r = 0.94$ The correlations between the pulse rates for 2 and 4 min are somewhat lower $r = 0.88$ and $r = 0.91$ respectively The re test correlations are between $r = 0.50$ and $r = 0.60$ for the 600 and $r = 0.60$ and $r = 0.70$ for the 900 kpm min levels

Table II The correlation coefficients for the pulse rates of the 1st and 2nd tests after 2 4 and 6 min cycling at 600 and 900 kpm min, and the correlations between these variables and the rating showing results (K)

N = 42. At r = 0.30 is P < 0.05 and at r = 0.39 is P < 0.01

	1	2	3	4	5	6	7	8	9	K
1 I 600 kpm min 2	—	—	—	—	—	—	—	—	—	– 17
2 I 600 kpm min 4	.83	—	—	—	—	—	—	—	—	– 14
3 I 600 kpm min 6	.85	.90	—	—	—	—	—	—	—	– 16
4 II 600 kpm min 2	.53	.51	.52	—	—	—	—	—	—	–.25
5 II 600 kpm min 4	.53	.59	.59	.91	—	—	—	—	—	–.31
6 II 600 kpm min 6	.59	.57	61	.91	.94	—	—	—	—	–.31
7 I 900 kpm min 4	—	—	—	—	—	—	—	—	—	–.36
8 I 900 kpm min 6	.80	[illegible]	8	.35	43	46	.97	—	—	–.35
9 II 900 kpm/min 4	—	—	—	—	—	—	.65	64	—	– 48
10 II 900 kpm min 6	.62	.62	66	4	6	.83	0	68	.53	–.51

Table III Correlation coefficients for values of physical working capacity according to PWC_{170}, estimated maximal O_2 uptake according to Åstrand with the averages (M) from the 1st (1) and 2nd (2) power levels pulse rate at 900 kpm min in the 1st (I) and the 2nd (II) tests, and showing results (K)

N = 42. At r = 0.30 is P < 0.05 and at r = 0.39 is P < 0.01

	1	2	3	4	5	6	7	8	9	10	11	K
1 I PWC_{170} 4	—	—	—	—	—	—	—	—	—	—	—	43
2 I PWC_{170} 6	.94	—	—	—	—	—	—	—	—	—	—	46
3 II PWC_{170} 4	63	3	—	—	—	—	—	—	—	—	—	.50
4 II PWC_{170} 6	71	6	9	—	—	—	—	—	—	—	—	.54
5 I Åstrand 1	—	4	—	0	—	—	—	—	—	—	—	.29
6 I Åstrand 2	—	91	—	79	83	—	—	—	—	—	—	.38
7 I Åstrand M	.86	85	9	.8	—	—	—	—	—	—	—	.35
8 II Åstrand 1	—	42	—	.6	.62	53	—	—	—	—	—	.56
9 II Åstrand 2	—	61	—	.91	63	1	—	79	—	—	—	45
10 II Åstrand M	.50	53	84	.81	—	—	67	—	—	—	—	42
11 I Pulse 900 6	– 9	– 81	– 6	8	–.81	–.9	– 94	–.54	– 69	– 63	—	– 35
12 II Pulse 900 6	– 52	– [illegible]	– 91	–.88	– 62	– 6	– 67	– 86	– 93	–.94	– 68	–.51

The correlations in Table III also reveal high reliability coefficients Thus the intra test correlations for the PWC_{170} values are r = 0 94 and r = 0 97 for the 1st and 2nd tests respectively The correlations between the Åstrand values within the tests are r = 0 83 and r = 0 79 These correlations must be considered high as they to a certain extent are based on the assessments of 2 power levels and could therefore be compared with the "correspor

Table IV Correlation coefficients between some morphological variables a criterion consisting of results from a skiing competition and some physiological variables

When r = 0 30 P < 0 05 and when r = 0 39 P < 0 01

	Chest width	Height	Body weight	Pulse rate 900 I	Pulse rate 900 II	PWC_1 I	PWC_1 II	Åstrand I	Åstrand II	C te on
Chest width	—	27	75	− 43	− 31	40	37	50	34	09
Height	—	—	56	− 16	− 20	04	15	15	20	03
Body weight	—	—	—	− 5[illegible]	− 29	45	36	55	32	24

correlations between the pulse rates at two power levels, which are r = 0 78 and r = 0 83 (Table II) The re test correlations for the PWC_{170} values from 5 minute assessments are as high as r = 0 76 The Åstrand values from the higher level on both occasions correlate r = 0 71, the averages for both power levels being r = 0 67 The correlations between the Åstrand values and the pulse frequencies (6 min) from 900 kpm/min range between r = − 0 92 and r = − 0 94 (Table III)

The validity correlations reveal a fairly good correspondence between several test measurements and the criterion The highest correlations obtained were those between the criterion and the different test measurements from the 2nd test which as regards time, was nearest the criterion and was performed approximately between 1 and 2 months later Thus the correlations between the pulse rates from 6 min work at 900 kpm/min in the 1st and 2nd tests and the criterion are r = − 0 35 and r = − 0 51 respectively The corresponding correlations between the criterion and the PWC_{170} values are r = 0 46 and r = 0 54 and between the criterion and the Åstrand values from the highest level r = 0 38 and r = 0 45

The averages (M) of the Åstrand values from the 1st and 2nd power level reveal no closer connection (but about the same) with the criterion than do the values from the highest level only

The inter variation between breathing frequencies has also been assessed. The intra test correlation has here been calculated between the frequencies after working periods of 3 and 5 min and shows a very close correspondence Thus the correlations at the 600-level on both occasions were about r = 0 90 and at the 900-level r = 0 95 The re test correlations are between r = 0 75 and r = 0 84 The correlation of the breathing frequencies with the pulse rates or the criterion however is throughout very low The best connection from 900 kpm/min was here obtained with the Åstrand values with negative correlations from about r = − 0 20 to r = − 0 30 (the latter scarcely significant, P < 0 05)

The correlations of the morphological variables chest width body weight and height with some physiological variables are seen by the coefficients in Table IV, which contains all the significant correlations There was thus no significant correlation between the morphological measures and the breathing frequency As seen from the Table the height shows no significant correlation with any of our physiological measures The highest correlations are seen between body weight and pulse rate or maximal oxygen uptake according to Åstrand with r = —0 55 and r = 0 55 respectively Body weight correlates also with PWC_{170} r = 0 45 Chest width shows about the same correlations as body weight though the coefficients are somewhat lower The best correlation with the criterion shows body weight r = 0 24 But this coefficient is not significant

No fair estimation of the reliability of the criterion can be made The correlation coefficient between both skiing races was r = 0 90 but this coefficient is rather uncertain as it is based only on the achievements of 15 participants

Discussion

The calculated measurements of reliability proved to be comparatively high this applies both to the different intra test correlations and to the re test correlations This reliability estimation can be compared to that of Linderholm (personal communication) who for a heterogeneous group of 18 men and women with a physical working capacity according to PWC_{170} ranging from 625 to 1 555 kpm/min found a re test correlation between two tests with 4 days interval of 0 97 thus establishing the high degree of reliability of this method. The difference between the re test correlations from Linderholm s study and ours shows that the influence on the soldiers of the military service from the summer of 1957 to the spring of 1958 has been acting in different directions Inter alia the physical working capacity has declined for some of the individuals with the highest initial capacity and increased for some of those with the lowest capacity

Naturally the different pulse measurements of 2 4 and 6 min work are not independent of each other as later measurements include previous ones plus an additional contribution The computation of the correlation between the different levels results in coefficients more independent of each other These correlations are in fact also very good as are the re test correlations which throughout reveal good reliability

A study of the differences between the correlations shows that the reliability seems to increase with a longer period of cycling from 2 4 to 6 min An increase also seems to take place from 600 to 900 so that a higher power level yields a higher reliability

The breathing frequencies appear to show the same tendency as the pulse frequencies The different reliability coefficients are very high As the breath-

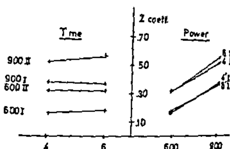


Fig 1 The variation in the validity coefficients (Fisher's Z) due to the time at constant power level and to the power at time kept constant

ing frequency may be voluntarily influenced the experimental subject's breathing attitude may have been conducive to constancy

At best the calculated validity correlations lie about r = 0 50 Judging from these the best test measurement appears to be the power at a pulse rate of 170 beats/min according to PWC_{170} The pulse rates from the highest power levels correlate almost as well with the criterion as do the power values As there was about one month between the skiing race and the second test the validity coefficient may have been lowered by this difference in time A still higher correspondence between work test and skiing race as shown by the validity correlation may therefore have been obtained if these two indicators physical working performance had been arranged with very short time al

There appears to be no correlative advantage in transposing the pulse frequencies to estimations of maximal O_2 uptake in accordance with the Åstrand nomogram except in the case of a low power level with a slightly positively skew distribution of the pulse rates when a transfer to O_2 values may bring about a certain normalization At a high power level when the distribution of the pulse frequencies may be influenced by the pulse ceiling the effect is however the opposite

The condition criterion used was a 20 mile (30 km) skiing race As this race formed part of the obligatory training the groups of participants were considered an unbiased sample On the other hand the incentive to good achievement may perhaps have been uneven The correlation coefficient with the later race of 10 miles (15 km) revealed a good connection It may be of interest to correct the correlation coefficients for attenuation (GUILFORD 1954) If the reliability coefficient of the criterion is r = 0 90 and the re-test reliability is r = 0 85 the validity coefficient will be r = 0 62 following the correction of erroneous measurements in both variables i e correction for attenuation If the criterion reliability is only r = 0 70 and the test reliability the same the validity coefficient will be r = 0 70

It was previously stated that the reliability coefficients were higher for the higher power level This is also the case with the validity coefficients. But the time increase from 4 to 6 min is not active in the same way as the

power increase In Fig 1 a graphic description is given of th ncrease of correlation both due to the time at a given power level and to the power at a given time. The correlation coefficients have been transformed to Fisher s Z-coefficients so as to give a better picture of the real difference in strength As will be seen the increase of power from 600 to 900 kpm/min is a power fully active factor whilst the time increase from 4 to 6 is not In the assessment of the working capacity of normal individuals 4 work appears to be sufficient. It may be suitable to increase the number of power levels at the cost of time for each level without prolonging the total test period

In spite of the good reliability of the breathing frequencies there was hardly any correlation at all or only a moderate one with the other values It is a wellknown fact however that the breathing frequency offers a serviceable measurement for purposes of diagnosis and consequently a knowledge of its reliability is of great interest In a heterogeneous group of experimental subjects including also patients with different diseases this reliability coefficient will probably be still greater

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From The University Institute for Experimental Medical Research
Ullevaal Hospital, Oslo, Norway

Urine Oxygen Tension, Lack of Correlation to Some Renal Functions

By

KNUT AUKLAND

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Abstract

AUKLAND, K. *Urine oxygen tension, lack of correlation to some renal functions.* Acta physiol. scand. 1962. 55. 362—375. — Urine oxygen tension (UpO_2) can be varied independently of oxygen tension of renal venous blood and renal blood flow by means of vasoactive drugs. It is assumed that UpO_2 is determined by pO_2 in the renal papilla by equilibration across the walls of the collecting ducts. UpO_2 might therefore be a function of the rate of blood flow to the medulla and papilla. To test this hypothesis 8 experiments were carried out in Nembutal anesthetized dogs. UpO_2 was recorded polarographically and was varied between 8 and 60 mm Hg by i. v. infusion of histamine, papaverine, adrenaline and nor adrenaline. UpO_2 showed no consistent correlation to T_{H_2O}, F_{PAH} or C_{Urea}/C_{Cr}, functions which might be expected to depend on the rate of medullary blood flow. The experiments thus gave no support for the hypothesis that the variations in UpO_2 were due to variations in medullary blood flow. No definite conclusion is permitted, however, because the dependency of these parameters on medullary blood flow has not been proved.

In a previous study it was shown that urine oxygen tension (urine pO_2) may be varied by vasoactive substances such as histamine, papaverine, adrenaline and nor adrenaline (AUKLAND and KROG 1961). The variations in urine pO_2 were not accompanied by parallel changes in renal blood flow or oxygen tension of renal venous blood. Since the availability of oxygen in the renal papilla was found to be low (AUKLAND and KROG 1960), it was suggested that urine pO_2 reflects the oxygen tension of the renal papilla by equilibration across the walls of the collecting ducts. Based on this concept it was furthermore suggested that the rate of medullary and papillary blood flow

might be an important factor in the determination of urine pO_2 The present experiments were designed to test this hypothesis by simultaneous estimation of urine pO_2 and some renal functions which would be expected to be influenced by alterations in medullary blood flow, i e renal concentration capacity, PAH (para amino hippurate) extraction ratio and back diffusion of urea from the tubules The data were also analyzed to find out if there exists any relationship between urine pO_2 and glomerular filtration rate, renal blood flow urine flow or tubular sodium reabsorption

It has recently become evident that urine is concentrated in the collecting ducts by water reabsorption due to a high osmolar concentration in the interstices of the renal papilla Although several hypotheses have been advanced to explain the creation of hypertonicity in the papilla (WIRZ 1953 BERLINER *et al* 1958, GOTTSCHALK and MYLLE 1959 ULLRICH KRAMER and BOYLAN 1961 KIIL and AUKLAND 1961), it is generally agreed that the counter current exchange effect of the vasa recta contributes to maintain the high osmolar concentration (WIRZ 1953) Increased flow in the vessel loops will reduce the efficiency of this counter current exchange mechanism and would therefore be expected to lower the papillary osmolar concentration and thereby reduce the amount of water which can be extracted from the collecting ducts It was therefore of interest to estimate the negative free water clearance (T_{H_2O}) during high osmotic diuresis at varying urine pO_2

It has been assumed by Reubi and coworkers (for references see discussion) that blood flowing through the juxtamedullary glomeruli is not cleared of para amino hippurate (PAH) because the efferent vessels of these glomeruli dip directly into the medulla without coming into contact with the PAH secreting proximal tubule cells Although this concept has been denied by other investigators it was of interest to find out if there exists any relationship between urine pO_2 and E_{PAH} (extraction ratio of PAH)

The high papillary osmolar concentration is made up chiefly by sodium chloride and urea (ULLRICH and JARAUSCH 1956) It is assumed that urea is provided to the papilla by diffusion from the collecting ducts which have been shown to be permeable to urea (KLÜMPER ULLRICH and HILGER 1958 KIIL and AUKLAND 1960 AUKLAND 1961) An increase of the flow rate in the vasa recta would be expected to remove more urea from the papilla and thereby increase urea loss from the collecting ducts An attempt was therefore made to correlate back diffusion of urea to urine pO_2

Methods

Eight experiments were performed on healthy mongrel dogs of either sex weighing 15—22 kg Anesthesia was induced by Nembutal sodium i 25 mg/kg body weight and maintained with subsequent doses of 1—2 mg/kg A tracheal tube was inserted to assure free air ways All experiments were performed with spontaneous breathing of air

The left ureter was exposed by a small flank incision and cannulated by a polyvinyl catheter which was then connected to the pO_2 measuring chamber. Urine pO_2 was recorded continuously by a teflon-covered electrode (KROG and JOHANSEN 1959) mounted in a plastic chamber as previously described (AUKLAND and KROG 1961). Urine was collected for analysis at the outlet of the electrode chamber. Urine from the other kidney was drained by an indwelling bladder catheter.

A polyethylene catheter was inserted into a brachial artery for continuous recording of arterial pressure by means of a Sanborn amplifier and recorder. A three way stop cock was interpositioned between the catheter and the strain gauge transducer allowing arterial blood to be collected from the same catheter. In three experiments the left renal vein was catheterized by a cardiac catheter of size 7 or 8 F, introduced through a jugular vein and the vena cava. Side branches to the renal vein were ligated. Clotting in the renal vein catheter was prevented by continuous infusion of a dilute heparine saline solution at a rate of 0.5 ml/min. The position of the catheter in the renal vein was checked at the end of each experiment.

All experiments were performed during high urine flow produced by a priming and sustaining infusion of mannitol in 0.45 % saline in order to minimize oxygen equilibration with systemic blood in the walls of the renal pelvis. High urine flow was also necessary for measurement of the negative free water clearance (4 experiments) and in these experiments 10 or 15 % mannitol was given at a rate of 8 ml/min. In the remaining four experiments 5 % mannitol was infused at the same rate. Creatinine (Cr) and sodium para amino hippurate (PAH) was added to the priming and sustaining infusion amounts to provide a constant plasma concentration within the following ranges: PAH 0.5—1.9 mg/100 ml, Cr 8—16 mg/100 ml. At high urine flow 0.1? [illegible] saline as given i.v. by drip at a rate adjusted to compensate for salt and water loss. After an equilibration period of at least 40 min, clearance periods of 5—10 minutes duration were started. Arterial blood was collected at the midpoint of each period. Renal extraction ratio of PAH (E_{PAH}) was estimated in three experiments. Systemic arterial and renal venous blood was collected simultaneously, immediately cooled in ice water and centrifuged at + 4 °C. Plasma was thus pipetted off within 8 min after sampling.

Histamine, papaverine, adrenaline and nor adrenaline were used to vary urine pO_2. The drugs were administered i.v. in 0.45 % saline with an electric infusion pump calibrated to deliver 0.25, 0.8 and 1.5 ml/min respectively. 89 clearance periods were performed without administration of drugs. Histamine was given in 10 periods of 15—65 min duration in 7 experiments at a rate of 8—60 µg/min. 52 clearance periods were performed during infusion of histamine. Papaverine was infused at a rate of 2—12 mg/min in three periods of 25—35 min duration including 14 clearance periods. Adrenaline and nor adrenaline were given in six experiments at a rate of 5—32 µg/min in periods of 15—25 min duration. Five clearance periods were carried out during adrenaline and 16 during nor adrenaline infusion. In two experiments clearance periods were carried out continuously throughout the whole experiment, while in the remaining experiments 5—15 min were allowed for equilibration following start and stop of drug infusion.

Creatinine was estimated by the method of BOSNES and TAUSSKY (1945) and PAH by the method of SMITH *et al.* (1945). Sodium and potassium were analyzed with a Baird flame photometer using internal Li standard. Urea in blood and urine and urinary ammonia were determined by the microdiffusion method of CONWAY (1933). Plasma and urine osmolarity was determined in 2 ml samples in a Fiske osmometer. Negative free water clearance ($T^c_{H_2O}$) was calculated as osmolar clearance less urine flow (C_{osm}—V) (WESSON and ANSLOW 1952). Systemic arterial hematocrit was determined in Wintrobe tubes spun for 30 min at 3 000 r.p.m.

Table I Effect of various drugs on urine oxygen tension PAH-clearance renal blood cell flow glomerular filtration rate tubular sodium reabsorption and urine flow See text for dosage

	Urine pO	C_{PAH}	RBCF	GFR and T_{Na}	V
Histamine	+	— 0	— 0+	— 0	— 0
Papaverine	+	— 0	— 0	—	—
Adrenaline	— +	—		— 0	+—
Nor adrenaline	— +	— +		+ 0	+

Increase or decrease related to adjacent control periods
+ = increase, 0 = unaltered, — = decrease

In addition to the experiments described above one experiment was performed to investigate whether histamine papaverine adrenaline and nor adrenaline influenced the oxygen tension of arterial blood The femoral artery of an anesthetized dog was cannulated and blood lead through a polarographic chamber as described for urine and then returned to the femoral artery Rapid single injections and continuous infusions of histamine papaverine adrenaline and nor adrenaline were given i v in the same doses as those employed in this and a previous study (AUKLAND and KROG 1961) both during oxygen and air breathing

Results

The effect of continuous i v infusion of histamine papaverine adrenaline and no-adrenaline on urine pO_2 reported previously (AUKLAND and KROG 1961) was confirmed in the present study Histamine and papaverine invariably increased urine pO_2 while the effect of adrenaline and nor adrenaline was variable Continuous recording of arterial pO_2 in one experiment failed to show any effect of these drugs on arterial pO_2 in the doses employed

Some renal effects of the drugs have been summarized in Table I and the effect of histamine is shown in more detail in Fig 1 Clearance of PAH(C_{PAH}) and renal blood flow (RBF) decreased (Fig 1) or remained unchanged during histamine infusion However due to a moderate increase of systemic hematocrit the flow of red cells through the kidney (RBCF) increased in some infusion periods Since the mean arterial pressure declined moderately during histamine infusion the total renal vascular resistance remained unchanged or decreased slightly Glomerular filtration rate (GFR = clearance of creatinine C_{Cr}) remained unchanged or decreased moderately Sodium and potassium excretion most often decreased during histamine infusion However the filtered load of sodium decreased more than sodium excretion resulting in a fall in tubular sodium reabsorption (T_{Na}) It should be noted that an increase of urine pO_2 during histamine infusion was sometimes observed also when GFR and T_{Na} remained unchanged and irrespective whether RBCF increased or decreased

Papaverine most often produced a moderate decrease of C_{PAH} RBCF GFR and T_{Na} while urine pO invariably increased

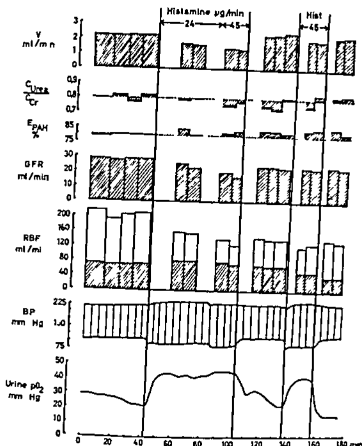


Fig 1 Effect of histamine on urine pO and other parameters Dog G ♀ 15 kg Priming infusion 50 min before start of clearance periods 10 g mannitol 1 g creatinine and 120 mg sodium para amino-hippurate in 100 ml 0 45 % saline Sustaining infusion 50 g mannitol 1.25 g creatinine and 350 mg Na PAH in 1 000 ml 0 45 % saline Infusion rate for both solutions 8 ml/min. Histamine given by continuous i v infusion at rates indicated at the top of the diagram. Hatched areas on renal blood flow columns (RBF) indicate renal blood cell flow

During adrenaline infusion C_{PAH} decreased markedly while GFR remained unaltered or decreased slightly Nor adrenaline had no consistent effect on C_{PAH} while GFR remained unaltered or increased slightly (RBCF was not estimated during adrenaline and nor adrenaline infusion) Both adrenaline and nor adrenaline had a varying effect on urine pO_2 in some infusion periods depressing in others increasing and quite often great fluctuations in urine pO_2 were obtained (In Fig 2—5 the mean urine pO_2 of each period has been used)

In most experiments there was a slow fall in C_{PAH} and GFR apparently in dependent of drug administration No consistent change in the filtration frac tion C_{Cr}/C_{PAH} was observed

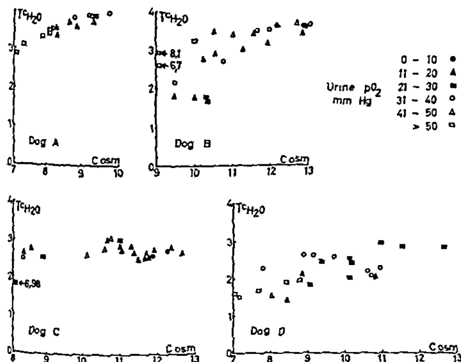


Fig 2 Illustrates lack of correlation between urine pO_2 and tubular reabsorption of solute free water (T_{H_2O}) at various levels of solute excretion in four experiments Tm_{H_2O} and Cosm in ml/min. Sustaining infusion of 10 or 15 % mannitol solution at a rate of 8 ml/min. Urine pO_2 varied by periods of continuous infusion of histamine, papaverine adrenaline or nor adrenaline. Dog A, ♀ 15 kg Dog B ♀ 15 kg Dog C, ♀ 19 kg Dog D ♂ 15 kg

Renal concentrating capacity and urine pO_2

The negative free water clearance (T_{H_2O}) varied considerably throughout each of 4 experiments Since the osmolar clearance (C_{Osm}) in several periods dropped beyond the value required to obtain maximal T_{H_2O} (according to PAGE and REEM (1952) 3 5 times Tm_{H_2O}) it is reasonable to correlate T_{H_2O} to C_{Osm} However as shown in Fig 2 the scattering of the values seems not to be explicable only by varying osmolar clearance The diagram shows furthermore that the variations in T_{H_2O} were not associated with changes in urine pO_2 in any consistent manner In three of the four dogs (B C and D) T_{H_2O} was better correlated to glomerular filtration rate (GFR) than to C_{Osm} as shown in Fig 3 The mean ratios between T_{H_2O} and GFR were Dog A 0 089, Dog B 0 091 Dog C 0 070 and Dog D 0 070 As is also shown in Fig 3 was no consistent association between urine pO_2 and negative free clearance at any level of GFR

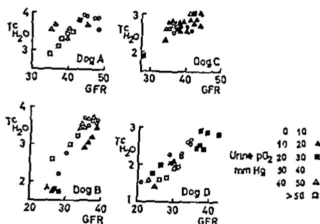


Fig 3 Lack of correlation between urine pO_2 and $T^c_{H_2O}$ at various levels of glomerular filtration rates. $T^c_{H_2O}$ and GFR in ml/min. Same experiments as in Fig 2. Note that in 3 experiments (B C D) is $T^c_{H_2O}$ better correlated to GFR than to C_{Osm} (the latter shown in Fig 2)

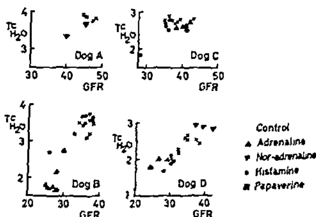


Fig 4 Illustrates lack of consistent effect of adrenaline, nor adrenaline histamine and papaverine on tubular reabsorption of solute free water. Same experiments as in Fig 2 and 3. $T^c_{H_2O}$ and GFR in ml/min

The correlation coefficients between $T^c_{H_2O}$ and C_{Osm} were Dog A + 0.95 Dog *B + 0.60* Dog C + 0.51 and Dog D + 0.69 and the correlation coefficients between $T^c_{H_2O}$ and GFR Dog A + 0.63 Dog B + 0.85 Dog C + 0.69 and Dog D + 0.91. However this statistical evaluation of the data suffers from two obvious sources of error. Since $T^c_{H_2O}$ (= V(U Posm − 1)) C_{Osm} (= V U/P_{Osm}) and GFR (= V U/P_{In}) all include the term V (urine flow) a false positive correlation may be expected. *Secondly if C_{Osm} is not high* enough to secure maximal $T^c_{H_2O}$ one would not expect *a straight line correlation* between C_{Osm} and $T^c_{H_2O}$ but rather an asymptotic approach *of $T^c_{H_2O}$ to its maximum*. However inspection of Figs 2 and 3 with this objection in mind shows that the rather large variations of $T^c_{H_2O}$ in Dogs B C and D are better explained by variations of GFR than of C_{Osm}. The negative free water clearance showed an equally high correlation to reabsorbed sodium as to GFR as would be expected from the fact that more than 85 % of the filtered sodium was reabsorbed in all periods.

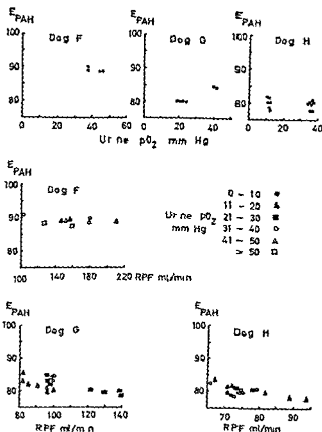


Fig 5 a Lack of correlation between urine pO_2 and renal extraction ratio of p-amino-hippurate b Lack of correlation between urine pO_2 and E_{PAH} also when variations in renal plasma flow are taken into account Arterial plasma concentrations of PAH between 0 58 and 1 99 mg/100 ml Dog F ♂ 17 kg Dog G ♂ 15 kg Dog H ♀ 17 kg

The data were also analyzed to find out if the infusion of drugs influenced the concentrating capacity It was found that adrenaline and nor adrenaline had no consistent effect on T_{H_2O} in the doses used while the mean T_{H_2O} during histamine infusion was lower than in control periods However when GFR is used as parameter (Fig 4) it seems unlikely that histamine has any specific effect on the concentrating mechanism apart from that exerted by reduction of GFR

Urine pO_2 and renal extraction of PAH

The renal extraction ratio of PAH (E_{PAH}) determined in 46 clearance periods in three experiments varied between 77 and 92 5 % The mean values in each experiment were (range given in parenthesis) Dog F 89 6 (88 5—92 5) % Dog G 81 9 (78 5—84 5) % and Dog H 80 0 (78—83 5) % In all three experiments the extraction ratio was found to be unrelated to urine pO_2 (Fig 5 a)

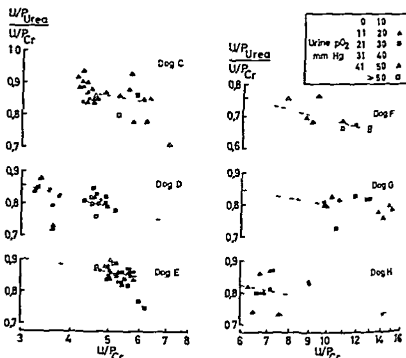


Fig 6 Lack of correlation between urine pO_2 and urea excretion ratio at various levels of urine flow Broken line drawn through observations and through $U/P_{Cr} = 1$ $U/P_{Urea}/U/P_{Cr} = 1$ (In dog C ammonia is included in urinary "urea" concentration)

It has recently been shown that E_{PAH} varies inversely with renal plasma flow (Harth Kreienberg and Lutz 1959) This effect might possibly mask a correlation between urine pO_2 and E_{PAH} In Fig 5 b E_{PAH} has therefore been plotted against renal plasma flow using urine pO_2 as a parameter However even when compared at a given rate of plasma flow, there was no consistent relationship between urine pO_2 and E_{PAH} As also shown in Fig 5 b variations in plasma flow had only slight effect on E_{PAH} in these experiments

Urine pO_2 and renal urea excretion

Simultaneous estimation of urea and creatinine clearance was carried out in 129 clearance periods in 6 dogs with urine oxygen tensions varying between 8 and 56 mm Hg Urea excretion ratio *i e* the ratio of filtered urea which is excreted in urine is given by the ratio C_{Urea}/C_{Cr} which is equal to $U/P_{Urea}/U/P_{Cr}$ Since urea excretion ratio is strongly dependent on urine flow (or U/P_{Cr}), the excretion ratio cannot be directly correlated to urine pO_2 In Fig 6 urea excretion ratio has been plotted against U/P_{Cr} using a semilogarithmic system (Shannon 1936) and different levels of urine pO_2 are indicated by the same symbols as used in previous figures The values show rather great scattering but it is evident that the variations bear no consistent relationship to urine pO_2

Discussion

In planning the experiments reported above it was hoped to establish a positive correlation between urine pO_2 and renal medullary blood flow. It was found, however, that none of the parameters used — renal concentrating capacity, PAH extraction ratio and tubular back diffusion of urea showed any consistent correlation to urine pO_2. This may mean either that urine pO_2 is not determined by medullary blood flow or it may indicate that none of the parameters used is dependent on medullary blood flow. The latter possibility will be discussed.

The function of the vasa recta in the renal medulla as a passive countercurrent diffusion system seems inevitable from the morphological characteristics of these vessels, and is also supported by experimental evidence (WIRZ 1953, GOTTSCHALK and MYLLE 1959). Furthermore, theoretical considerations show that the flow rate in the vasa recta must influence the efficacy of the exchange mechanism in such a way that an increased flow would reduce the efficacy in maintaining a high osmolar concentration within the papilla (HARGITAY and KUHN 1951, BERLINER *et al.* 1958). It is possible, however, that other factors, such as sodium load to the loops of Henle may be more critical in the determination of hypertonicity in the papilla (LEVINSKY, DAVIDSON and BERLINER 1959). Furthermore, it can not be regarded as proved that osmotic equilibrium is established across the walls of the collecting ducts even in the presence of antidiuretic hormone. If this were not the case, T_{H_2O} will not reflect the capacity of the concentrating mechanism, as pointed out by LEVINSKY and BERLINER (1959), who found positive free water clearance even during high solute diuresis. In the present experiments, however, the negative free water clearance remained high throughout all experiments at all levels of solute excretion, and the mean values of T_{H_2O}/GFR are equal to or even higher than those reported as Tm_{H_2O}/GFR by other investigators (PAGE and REEM 1952, ANSLOW and WESSON 1955, BRICKER *et al.* 1960).

It must be concluded that the concentration mechanism must be dependent on the flow rate in the vasa recta, but other factors may be of greater significance. Furthermore, it cannot be ascertained that T_{H_2O} gives a correct measurement of the capacity of the concentration mechanism. The lack of correlation between T_{H_2O} and urine pO_2 therefore does not exclude the possibility that urine pO_2 is mainly determined by medullary blood flow.

If the concentration process in the medulla depends upon the load of sodium to the loops of Henle, it would be expected to be influenced by the glomerular filtration rate, and especially the filtration rate of juxtamedullary nephrons having long loops. It was found that T_{H_2O} showed better correlation to GFR than to C_{Osm}. It is therefore conceivable that the filtration rate of juxtamedullary glomeruli has varied parallelly with the measured total GFR, which probably mainly reflects the filtration of cortical glomeruli because of

far greater number The relatively great variations in GFR observed in these prolonged experiments thus seem to affect both cortical and juxtamedullary nephrons

BLAKE (1961) found recently that infusion of nor adrenaline reduced the negative free water clearance at any level of C_{Osm} and concluded that nor adrenaline may increase medullary blood flow at the expense of cortical nephrons Neither adrenaline, nor adrenaline nor histamine had any consistent effect on T_{H_2O} apart from that associated with alterations in GFR It should be noted however, that the experience with nor adrenaline is limited to 5 clearance periods in 2 dogs

The concept that PAH extraction ratio is determined by blood flow through the medulla has been advocated especially by Reubi and collaborators They assumed that plasma leaving the juxtamedullary glomeruli does not come in contact with proximal tubular tissue and is not cleared at all for PAH while cortical plasma is cleared 100 % If this assumption were correct the medullary plasma flow would be given by total renal plasma flow minus C_{PAH} Based on this hypothesis the effect of a great number of drugs has been investigated with the conclusion that cortical and medullary plasma flow may be varied independently (REUBI and SCHROEDER 1949 REUBI and FUTCHER 1949 REUBI [illegible] 0, 1958) No consistent effect of adrenaline or histamine on E_{PAH} was found [illegible] agreement with the present study It should be noted, however that the [illegible] is based wholly on anatomical considerations and not on experimental evidence and that other authors have arrived at directly opposite conclusions MAXWELL BREED and SMITH (1950) and SMITH (1951) have maintained referring to PETERS (1909) and TRUETA *et al* (1947) that in the outer medullary zone the vasa recta are intermingled with the descending straight limb of the proximal tubule probably capable of removing PAH also from the vasa recta They therefore believe that PAH is extracted from medullary blood as well as from cortical blood and furthermore that the uncleared fraction (1 E_{PAH}) represents blood supplying the renal pelvis capsule and perirenal fat The dependency of PAH extraction on renal plasma flow has recently been explained by KIIL (1961) in terms of enzyme kinetics without excluding that a small portion of the renal plasma flow may pass uncleared through the medullary circulation

At the present time the question whether E_{PAH} depends on medullary blood flow remains unsettled and can probably only be decided by micro puncture studies

The dependency of tubular back diffusion of urea on medullary blood flow may be even more questionable than that of T_{H_2O} and E_{PAH} It has been generally accepted that the high interstitial urea concentration in the renal papilla is derived from the collecting ducts by passive diffusion (for references see AUKLAND 1961) The passive counter exchange mechanism of the vasa recta will minimize the loss of urea from the papilla via the blood stream. Increased

flow rate in the vessels will reduce the efficiency of the counter current system and tend to wash out urea from the papilla This in turn should increase urea diffusion out from the collecting ducts and thereby reduce the urea excretion ratio It seems reasonable to assume however that in dogs on mixed diet the main urea back diffusion occurs from the convoluted tubules and that variations in urea loss from the collecting ducts are too small to produce measurable alterations in the final excretion ratio

Furthermore since the estimation of urea excretion ratio depends on four chemical determinations each with their sources of error a rather great scattering of the values would be expected It is conceivable however that the more extreme variations seen in Fig 6 may be due to inadequate equilibration resulting in falsely high or low urea clearances due to washout or accumulation of urea in the papilla (For references see AUKLAND 1961)

The above discussion may be summarized as follows No consistent correlation could be demonstrated between urine pO_2 and T_{H_2O} E_{PAH} and C_{Urea}/C_{Cr} respectively but unfortunately none of these parameters has been undisputably proved to depend upon medullary blood flow The results therefore permit no definite conclusion as to whether urine pO_2 is mainly determined by the rate of medullary blood flow or not

In the preceding discussion it has been referred to medullary and papillary blood flow as if these would always vary parallelly This may be an unjustified simplification Both the outer and inner medullary zone (the latter comprising the papilla) derive their blood supply from the efferent vessels of juxtamedullary glomeruli via the vasa recta However independent variations of blood flow in outer and inner medulla might occur and evidence for red cell shunts in the outer medulla has also been presented (THURAU SUGIURA and LILIENFIELD 1960) This might influence urine pO_2 E_{PAH} T_{H_2O} and $U/P_{Urea}/U/P_C$ in different and unpredictable ways

We have previously suggested that variations in medullary oxygen consumption may be an important factor in determining urine pO_2 (AUKLAND and KROG 1961) If medullary oxygen consumption is mainly concerned with sodium transport as seems to be the case for the kidney as a whole (KRAMER and DEETJEN 1960 LASSEN MUNCK and THAYSEN 1961 KIIL AUKLAND and REFSUM 1961) the load of sodium to the medulla might be of importance Some observations in the present study support this concept A rise in urine pO_2 was most often accompanied by a fall in GFR and sodium filtration On the other hand rapid i v infusion of hypertonic mannitol glucose or urea reduces urine pO_2 (AUKLAND and KROG 1961) This might be due either to the increased amount of sodium delivered from the proximal tubules increasing medullary sodium transport or to a more expensive sodium reabsorption in the presence of large amounts of non reabsorbable solutes (KIIL AUKLAND and REFSUM 1961) A similar effect of hypertonic sodium chloride might favor the former explanation Furthermore in unpublished experiments

it has also been shown that urine pO_2 is lowered by mercurial diuretics. Although an increased oxygen consumption seems unlogical in the presence of a transport inhibitor, it might be due to a preferential effect of mercurials in the proximal tubules, increasing the amount of sodium delivered to the loops of Henle, thereby increasing sodium reabsorption and O_2 consumption in this place.

Although medullary sodium transport itself cannot be measured *in vivo*, it is hoped that further studies along this line may provide an explanation for the surprisingly low and variable oxygen tension of urine.

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From the University Laboratory of Physiology Oxford, England

The Effects of Fusimotor Activity on the Static Responsiveness of Primary and Secondary Endings of Muscle Spindles in the Decerebrate Cat

By

J K S JANSEN[1] and P B C MATTHEWS

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Abstract

JANSEN J K S and P B C MATTHEWS *The effects of fusimotor activity on the static responsiveness of primary and secondary endings of muscle spindles in the decerebrate cat* Acta physiol scand 1962 55 376—386 — The effect of fusimotor activity on the static behaviour of soleus muscle spindle receptors in the decerebrate cat has been investigated by comparing their responses before and after ventral root section The endings were classified as primary or secondary according to the conduction velocities of their afferent fibres Their static behaviour was assessed by measuring their frequency of discharge at different lengths 0 5 sec after completion of a slow stretch at 3 mm/sec Fusimotor activity increased the static responses of both kinds of ending measured at physiological full extension the average increase was slightly greater for primary than for secondary endings The static responses of both kinds of ending tended to increase or decrease together when the level of fusimotor activity changed The static sensitivity (slope of frequency extension relation) of both kinds of ending was commonly but not invariably increased by fusimotor activity These findings in conjunction with previous work, show that the behaviour of primary and of secondary endings is more alike under static than under dynamic conditions This is discussed in relation to the possible functions of the secondary ending

The primary and secondary afferent endings of muscle spindles are differently arranged with respect to the intrafusal muscle fibres (RUFFINI 1898 BARKER 1948 BOYD 1961) and might be expected to be differently affected

[1] Fellow of the Rockefeller Foundation. Present address Laboratory of Neurophysiology Department of Anatomy University of Oslo Norway

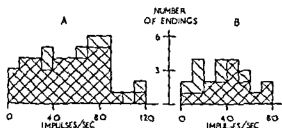


Fig 1 The effect of spontaneous fusimotor activity on the static responses of 45 primary endings (A) and 22 secondary endings (B) The abscissa is the increase in static response above that of the de efferented ending produced by fusimotor activity The static response was measured with the muscle at physiological full extension When the level of fusimotor activity varied during the experiment the largest increase observed in the static response has been plotted (☒ observations made in preparations with ipsilateral dorsal roots cut ☐ observations made with dorsal roots L7 and S1 intact.)

by their contraction A preceding paper (JANSEN and MATTHEWS 1962) has described some effects of the fusimotor activity occurring in the decerebrate cat on the dynamic responses of these endings to slow stretch of the muscle The present paper based on observations from the same series of experiments compares the effects of fusimotor activity on the static responses of the two kinds of ending to maintained extension

Methods

The methods used have already been described (JANSEN and MATTHEWS 1962) In outline they were as follows Fourteen cats were decerebrated by mid brain section under ether anaesthesia The discharge of single afferents from muscle spindles in the soleus muscle was recorded from dorsal root filaments The endings were classified as primary or secondary endings on the basis of the conduction velocities of their afferent fibres (cf HUNT 1954) The response of the endings was observed during and immediately after stretching the muscle at a slow constant velocity of about 3 mm/sec the final extension corresponded to full physiological extension as determined while the soleus tendon was still attached to the calcaneum This was done both when the motor supply of the endings was intact and again after they had subsequently been de efferented usually by ventral root section but occasionally by curarization In all but four of the experiments the appropriate dorsal roots were cut at the beginning of the experiment in order to simplify interpretation of the results A few results obtained with intact dorsal roots have been included in the present series and suggest that the present results are also applicable to fully innervated endings any result described is however from a de-afferented preparation unless otherwise stated The responses of the endings to the stretches were compared by measuring their mean frequencies of discharge over periods of 0 1 sec

Results

The afferent discharge from a muscle spindle evoked by stretching the muscle containing it may be considered to consist of two parts a static response related to the amount of extension and a dynamic response related to the rate of extension When the muscle is stretched and then kept extended the dynamic response of any spindle ending disappears on completion of th

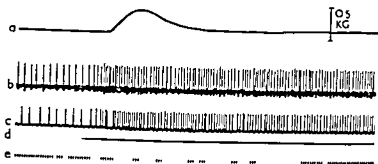


Fig 2 The responses of a primary and of a secondary ending recorded simultaneously during a crossed extensor reflex in soleus a isometric tension b discharge of secondary ending c discharge of primary ending d signal light indicating stimulation of contralateral popliteal nerve at 18/sec e 1/100 sec time marks (Initial tension 50g length 9 mm short of physiological full extension Conduction velocities of afferent fibres 46 and 97 m/sec for b and c respectively Dorsal roots cut ipsilaterally but not contralaterally)

dynamic phase of stretching leaving the static response The disappearance of the dynamic response is not however instantaneous and it may be many tens of seconds until a completely steady discharge is reached a phenomenon more familiarly known as adaptation Measurement of the final static response to an extension should therefore be made long after completion of the dynamic phase of stretching But this restricts the possible scope of experiments, for [illegible] of fusimotor fibres cannot necessarily be maintained at a constant level for prolonged periods and it also risks damage to the endings through the effects of prolonged tension (MATTHEWS 1933) However, a large part of the dynamic response decays in the first half second after completion of the extension the subsequent decay being much slower (JANSEN and MATTHEWS 1962) from the point of view of reflex function this early decay may be of more importance than that occurring over many seconds We therefore wish to present results on the static behaviour of endings assessed by measurements made 0 5 sec after completion of stretching even though the results obtained by measurement at a later time might be different

The effect of fusimotor activity on the static response of the endings was assessed in the first place by applying a standardized slow stretch (14 mm at 3 mm/sec terminating at physiological full extension) to the muscle both before and after interruption of its motor supply All the endings so studied whether primary (45) or secondary (22) had a smaller static response at the final extension after de efferentation than before showing that in the decerebrate cat both kinds of endings are tonically excited by spontaneous fusimotor activity The relative magnitudes of the effects of this spontaneous fusimotor activity on primary and secondary endings are shown in Fig 1 The variation for endings of one kind is probably due mainly to differences in the amount of fusimotor activity influencing them for the results are derived from 14

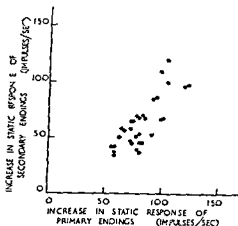


Fig 3 Comparison of changes in static responses of primary and secondary endings at different levels of fusimotor activity Observations from 5 primary and 4 secondary endings in the same experiment. The endings were recorded from in pairs consisting of a primary and a secondary ending For each pair the increase in static response at full extension of the secondary ending above its de efferented value has been plotted against the corresponding value for the primary ending (Mean de efferented static response of the primary endings 36 impulses sec range 26—48 impulses/sec of the secondary endings 51 impulses sec range 44—56 impulses/sec.)

different preparations The mean increase for the primary endings was 55 impulses/sec (S E of mean 4 4) while that of the secondary endings was 38 impulses/sec (S E of mean 4 1) The difference between these values is statistically significant (at 1 % level on t test) but in the present state of knowledge the finding that fusimotor activity increases the static responses of both kinds of endings by the same order of magnitude seems of more interest than this relatively small difference

This conclusion is supported by the finding that when in any one experiment the level of fusimotor activity changed the static responses of both primary and secondary endings tended to be affected similarly An example is shown in Fig 2 which displays the responses of a primary and of a secondary ending recorded simultaneously on eliciting a crossed extensor reflex in soleus by stimulating the contralateral lateral popliteal nerve After an initial burst their discharge settled down to an approximately constant level and the increase in maintained discharge was similar for the two endings being 55 impulses/sec for the primary ending and 50 impulses/sec for the secondary ending

Fig 3 shows a more systematic comparison of the relative effects on the two kinds of ending of changing levels of fusimotor activity in a single experiment Each point is derived from simultaneous observations on a primary and on a secondary ending as in Fig 2 The increase in the static response of the secondary ending above its de efferented level as measured after a standard stretch is plotted against the corresponding value for the primary ending The points are derived from repeated observations on several pairs of endings each observed at different levels of fusimotor activity The changes in fusimotor activity either occurred spontaneously or were produced reflexly sometimes by eliciting a crossed extensor reflex in soleus and sometimes by galvanic stimulation of the labyrinth (see JANSEN and MATTHEWS 1962) It

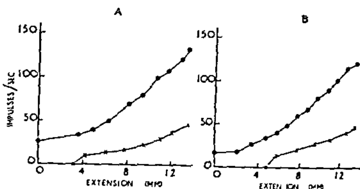


Fig 4 Static frequency-extension curves of a primary ending (A) and a secondary ending (B) determined during spontaneous fusimotor activity ( ) and after section of ventral roots ( ) Both endings were in the same preparation (Conduction velocity of afferent fibres 110 and 52 m/sec for A and B respectively)

is clear that in general the static responses of the two kinds of ending tended to increase together

Fig 3 is slightly more complicated than might appear at first sight because the arrangement into pairs of the 5 primary endings and 4 secondary endings was altered several times in the course of the experiment Some of the scatter of the points represents the effects of altering the pairs rather than the level of fusimotor activity but altering the pairs was not the cause of the correlation between the static responses of the two kinds of ending

Apart from the similarity in the increase of static discharge produced in primary and secondary endings by fusimotor activity there was also often a close correspondence between the actual rates of discharge of the two kinds of ending This can be seen directly for the pair of endings in Fig 2 and was also shown by statistical comparison of the whole series of endings of Fig 1 When their motor supply was intact the mean static response at full extension was 96 impulses sec (S D 35) for the primary endings and 80 impulses sec (S D 26) for the secondary endings When they were de efferented the values were 41 impulses sec (S D 18) for the primary endings and 42 impulses sec (S D 10) for the secondary endings

Effect of fusimotor activity on spindle static sensitivity

So far only the static response to a 14 mm stretch terminating at physiological full extension has been considered Observations of the static response at a number of other lengths were made by applying a slow stretch in a succession of small steps with short intervals in between during which the static response was determined again measured 0 5 sec after the completion of the dynamic phase of each small stretch) Fig 4 shows examples of frequency-extension relations so obtained both before and after de-efferentation for a primary ending and for a secondary ending The discharge frequency of each ending

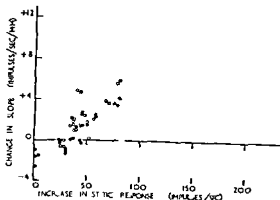


Fig 5 Scatter diagram relating changes in static sensitivity to increases in static response Data from 21 primary endings ( ) and 15 secondary endings ( ) studied systematically in this respect Abscissa, the increase in static response at full extension above its de efferented value produced by fusimotor activity Ordinate corresponding change in spindle static sensitivity produced by fusimotor activity (i e slope of static frequency-extension relation of innervated ending minus that of same ending when de efferented) The observations were made partly during spontaneous fusimotor activity and partly during reflexly induced changes in fusimotor activity They were all obtained from preparations in which the dorsal roots had been cut.

increased approximately linearly with extension over most of the range studied (cf ELDRED GRANIT and MERTON 1953 HARVEY and MATTHEWS 1961b) The slope of this line may be defined as the sensitivity of the ending to static extension (WHITTERIDGE 1959) and is of interest for theories on the servo-control of movement (cf JANSEN and MATTHEWS 1962) When de efferented both endings had a static sensitivity of about 4 impulses/sec/mm and fusimotor activity increased this to about 10 impulses/sec/mm in both cases

Fig 5 shows the change in static sensitivity of a series of endings related to the level of fusimotor activity the latter being judged by its effect on the static response at physiological full extension There is of course an element of tautology in this presentation since an increase of slope leads to an increase in the value of the final static response None the less the scatter diagram serves to show that fusimotor activity commonly increases the static sensitivity of both primary and secondary endings It can be seen for both kinds of ending however that the static response sometimes increased markedly without being associated with a simultaneous increase of slope (sensitivity) this was more common for primary than for secondary endings

When the endings of Fig 5 were de-efferented the mean value of the static sensitivity of the 15 secondary endings (3 6 impulses sec mm S D 0 9) was similar to that of the 21 primary endings (3 7 impulses/sec/mm, S D 1 3) These values are both slightly lower than those found by HARVEY and MATTHEWS (1961b) for a smaller series The explanation of this difference may be that in the earlier experiments the extension frequently terminated slightly beyond physiological full extension whereas in the present experiments the final extension was fairly close to physiological full extension The frequency extension relation although approximately linear over much of its range tends to be concave upwards, so that a greater mean value of slope would be found for large final extensions than for small final extensions, as the slope was measured over the last half to two-thirds of the range studied It should be noted also that not all frequency-extension relations are as linear as those shown in Fig 4

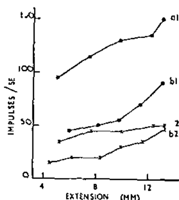


Fig 6 Decrease in static sensitivity of a primary ending accompanied by increase in its dynamic sensitivity Lower two curves ( ) show static frequency-extension relations of ending while its motor supply was intact (a2) and after subsequent de efferentation (b2) Upper two curves ( ) show dynamic frequency extension relation obtained during the dynamic phases of the same interrupted slow stretches (at 3 mm/sec) used to obtain a2 and b2 (Conduction velocity of afferent fibre 89 m/sec)

The changes in slope shown in Figs 4 and 5 cannot be attributed to contraction of extrafusal muscle fibres rather than to that of intrafusal muscle fibres Extrafusal fibre contractions were usually absent since the dorsal roots were cut at the beginning of all these experiments Contraction of extrafusal fibres however sometimes occurred during crossed extensor reflexes (cf Fig 2) Mechanical considerations suggest that they should have little effect on the sensitivity of the endings to stretch and this view was supported by comparison of the slopes of the frequency extension relations obtained with the stimulus to the contralateral nerve just above and just below the threshold eliciting a reflex contraction of the extrafusal fibres of soleus (the threshold for fusimotor effects was considerably lower)

Fig 5 shows also that the static sensitivity of primary endings was sometimes decreased by fusimotor activity It is interesting that this decrease in static sensitivity was usually accompanied by a definite increase in the dynamic response of the ending to the dynamic component of stretching An example of such behaviour is shown in Fig 6 The two lower curves (a2 b2) are the static frequency extension relations of the ending obtained by interrupted slow stretches (as Fig 4) applied when the ventral roots were intact (a2) and after they had been cut (b2) The two upper curves (a1 b1) are the responses obtained during the dynamic phases of the successive short slow stretches comprising the interrupted stretch The amount by which the curves obtained under dynamic conditions lie above their respective curves obtained under static conditions is a measure of the dynamic sensitivity of the ending to the slow stretch Clearly this dynamic sensitivity was increased by fusimotor activity at the same time as the static sensitivity was reduced Other observations made on the same ending at a different level of fusimotor activity showed a slight increase in slope of the static curve above the de efferented value accompanied by little change in the dynamic response from its de-efferented value Such observations suggest that the static sensitivity of primary endings depends upon more than one factor and that these factors have different effects on the dynamic sensitivity At any rate the effect of fusimotor activity on

the static behaviour of soleus spindle receptors cannot be expressed solely in terms of changes in their static sensitivity (cf HARVEY and MATTHEWS 1961) as has been possible for spindles in eye muscles (WHITTERIDGE 1959)

Discussion

The effects of physiologically maintained fusimotor activity on the static behaviour of primary and of secondary endings have proved to be similar in many respects This contrasts with the large differences described earlier in the effects of fusimotor activity on their dynamic responses (JANSEN and MATTHEWS 1961 1962) It was then argued that the dissimilarity in dynamic behaviour of the two kinds of ending occurs because the primary ending has large receptor terminals on the nuclear bag intrafusal muscle fibres whereas the terminals of the secondary ending are mainly on the nuclear chain intrafusal fibres (BOYD 1961) The similarity of static behaviour of the two kinds of ending would be explained if the receptor terminals which both kinds of ending have on the nuclear chain intrafusal muscle fibres were similarly influenced by contraction of these fibres If contraction of the nuclear chain fibres increased the stiffness of their polar regions relative to that of their receptor regions then the static sensitivity of the endings would be increased as here frequently found The decrease of static sensitivity which was sometimes observed for the primary endings may be tentatively attributed to contraction of the nuclear bag fibres since it was usually accompanied by an increase in the dynamic sensitivity of the endings (cf JANSEN and MATTHEWS 1962) Detailed speculation about the mechanism of these effects does not however appear profitable on the basis of the present work in which the relative amounts of contraction of the two kinds of intrafusal muscle fibre was unknown and in which the behaviour of primary and secondary endings was correlated statistically instead of by the more crucial method of comparing the behaviour of a pair of endings lying in the same muscle spindle (BESSOU and LAPORTE 1961)

In any detailed assessment of the present findings it should be remembered that they are based on measurements made 0 5 sec after completion of the dynamic phase of stretching at which time the endings had not adapted completely As however adaptation was then proceeding slowly similar results would almost certainly have been obtained if the measurements had been made one or two seconds later (as was checked in a few cases) but measurements at much later times might well give different results Half a second of rest is however a long time from the point of view of many reflex functions so the results obtained at this time appear to be of equal physiological interest to those obtained after fairly complete adaptation requiring perhaps 30 sec of rest The present results therefore provide a reasonable basis for discussing

the static responsiveness of primary and secondary endings but make no claim to be comprehensive in this respect

Taking a broader view the present results support the hypothesis suggested by all recent work that the fundamental functional difference between primary and secondary endings lies in their dynamic rather than in their static behaviour In comparison with secondary endings, primary endings give a much larger response to the dynamic component of an extension under all the conditions so far studied (COOPER 1959 1961 LUNDBERG and WINSBURY 1960 BESSOU and LAPORTE 1961 personal communication HARVEY and MATTHEWS 1961b) Further the dynamic sensitivity of primary endings can be altered over a wide range by fusimotor activity while that of secondary endings is little altered under similar conditions (JANSEN and MATTHEWS 1962) In contrast the differences in static behaviour of the two kinds of ending, though doubtless of functional significance appear relatively slight When they are de efferented the threshold tension (or extension) required to elicit maintained firing from primary endings is lower than that for secondary endings (HUNT 1954) and at short lengths of the muscle, primary endings are more strongly excited by fusimotor stimulation than are secondary endings (BESSOU and LAPORTE 1961, personal communication HARVEY and MATTHEWS 1961 a) In the present work fusimotor activity produced on average a slightly greater effect on primary than on secondary endings and this difference would probably have been more marked if systematic measurements had been made at shorter lengths of the muscle (for example compare Figs 4B and 6) But the static responses of the two kinds of ending were often similar both in their absolute frequency of discharge and in their static sensitivity All of this may be summarized to a first approximation by saying that while the primary ending signals both the length and the rate of change of length of the muscle the secondary ending signals mainly length with a sensitivity not very different from that of the primary ending Thus the difference in the frequency of discharge of the two kinds of ending approximately signals the rate of change of length of the muscle and it is easy to imagine that such a computation might usefully be performed by a nervous centre controlling movement Thus the difference in dynamic responsiveness of primary and secondary endings though as yet not fully explored appears to be a teleologically sufficient reason for the existence of two different kinds of sensory ending within the muscle spindle

Such an approach encourages further investigation of the reflex and central effects of the afferent discharges from secondary endings The present position is that electrical stimulation of group II muscle afferents presumed to be from secondary endings, reflexly excites flexor motoneurones and inhibits extensor motoneurones and that this occurs whether the afferent fibres stimulated originate in flexor or in extensor muscles (LLOYD 1943 BROCK ECCLES and RALL 1951 ECCLES R M and LUNDBERG 1959a) The same reflex pattern

produced by pulling on a muscle after blocking its group I fibres (LAPORTE and BESSOU 1959) On these and other findings the fibres from secondary endings have been classified as part of a widespread system of flexor reflex afferents also containing group III muscle afferents high threshold joint afferents and cutaneous afferents (ECCLES R M and LUNDBERG 1959b HOLMQUIST LUNDBERG and OSCARSSON 1960 LUNDBERG and OSCARSSON 1960) It seems unlikely however that the production of a general flexor reflex should be the sole use made by the central nervous system of the information provided by the secondary endings on the length of the muscle The present work shows that under static conditions the total flow of impulses from the secondary endings in a muscle may be comparable in magnitude to that from its primary endings for the two kinds of ending are approximately equally numerous (HUNT 1954) It seems probable therefore that secondary endings as well as primary endings play an essential part in the nervous control of muscle performance

We wish to thank Mr E T GILES for techn cal assistance.

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